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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

723K PCCODE 128857

MEMORANDUM

SUBJECT: 707-210 through 212, 707-215, 707-221, 707-EEE, 707-EGE. Myclobutanil (Rally®, Nova®, Eagle®). Conditional Registration Follow-Up Data Requirements: Review of Carcinogenicity Studies in Rats and Mice

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Pamela M. Hurley 7/12/94

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for 7/19/94

Background and Request:

Myclobutanil has been registered on a conditional basis pending submission of new carcinogenicity studies in male and female rats and in female mice at a higher dose level. The new studies have been provided by the Registrant, Rohm and Haas Company in hopes of obtaining unconditional registrations for the fungicide on the multiple crops which are currently registered on a conditional basis (MRID Nos. 428091-02 for the mouse study and 428091-01 for the rat study).

The Toxicology Branch (TB-I) has been asked to review the 2 carcinogenicity studies, determine whether or not they satisfy the regulatory requirements for carcinogenicity studies in 2 rodent species (83-2) and state whether or not there is an objection to changing the conditional registrations to unconditional registrations.

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Toxicology Branch Response:

TB-I has reviewed the 2 carcinogenicity studies in rats and mice and has determined that when combined with the previous chronic feeding/carcinogenicity study in rats and carcinogenicity study in mice (MRID Nos. 00165247 and 00164990, respectively), they satisfy the regulatory requirements for a carcinogenicity study in mice and a combined chronic feeding/carcinogenicity study in rats (83-2 in mice and 83-5 in rats). The combined rat studies are classified as Core Guideline and the combined mouse studies are classified as Core Minimum.

TB-I has no objection to changing the conditional registrations for myclobutanil to unconditional registrations. The toxicology data base is now complete.

Myclobutanil was reviewed by the RfD Committee on April 28, 1994. The RfD is currently established to be 0.025 mg/kg/day based on the NOEL from the chronic rat feeding study of 2.49 mg/kg/day and a safety factor of 100. The Committee classified myclobutanil to be a category E carcinogen: not carcinogenic in animal studies.

Myclobutanil was reviewed by the Less-than-Lifetime Committee on July 12, 1994. An acute dietary risk assessment is not required because an appropriate toxicological endpoint could not be found. A risk assessment for short term occupational or residential exposure (1 to 7 days) is required. The endpoint and dose to be used in risk assessment are from the 28-day dermal study conducted on the 40WP and the 2EC formulations. The NOEL's were both greater than 100 mg a.i./kg/day (highest dose tested). Therefore, 100 mg/kg/day will be used as the NOEL for the risk assessment. A risk assessment for intermediate term occupational or residential exposure (1 week to several months) is required. The endpoints and dose to be used in risk assessment are from the 2-generation reproduction study conducted on the technical material. The reproductive/developmental NOEL is 10 mg/kg/day and the LEL is 50 mg/kg/day based on an increased incidence in the number of stillborns, atrophy of the testes and prostate and a decrease in pup body weight gain during lactation.

In preparation for the RfD Committee meeting, some of the Data Evaluation Records (DER's) were updated and re-evaluated. As a result, some of the NOEL's and LEL's have changed. The updated DER's are attached to this memorandum along with the DER's from the two new carcinogenicity studies. The changes in the NOEL's and LEL's are as follows:

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Study	Old NOEL's, LEL's	New NOEL's, LEL's
Developmental Toxicity: Rabbit	Maternal NOEL: 20 mg/kg/day Maternal LEL: 60 mg/kg/day	Maternal NOEL: 60 mg/kg/day Maternal LEL: 200 mg/kg/day
Developmental Toxicity: Rat	Maternal NOEL: 312.6 mg/kg/day Maternal LEL: 468.9 mg/kg/day Developmental NOEL: 31.3 mg/kg/day Developmental LEL: 93.8 mg/kg/day	Maternal NOEL: 93.8 mg/kg/day Maternal LEL: 312.6 mg/kg/day Developmental NOEL: 93.8 mg/kg/day Developmental LEL: 312.6 mg/kg/day
Reproduction - 2 Generation: Rat	No developmental NOEL or LEL	Developmental NOEL: 200 ppm Developmental LEL: 1000 ppm
Carcinogenicity Study: Mouse (MRID No. 00164990)	Systemic NOEL: 20 ppm (2.7 mg/kg/day (♂), 3.2 mg/kg/day (♀)) Systemic LEL: 100 ppm (13.7 mg/kg/day (♂), 16.5 mg/kg/day (♀))	Systemic NOEL: 100 ppm (13.7 mg/kg/day (♂), 16.5 mg/kg/day (♀)) Systemic LEL: 500 ppm (70.2 mg/kg/day (♂), 85.2 mg/kg/day (♀))

The following paragraphs summarize the results of the new and old carcinogenicity studies in mice and the combined chronic feeding/carcinogenicity studies in rats. In addition, new summaries were provided for the studies mentioned in the above table and the 1-year feeding study in the dog. These are also included here. After the summaries, a Toxicology Profile is provided.

Chronic Feeding/Carcinogenicity Studies in the Rat

In a two year carcinogenicity study, technical myclobutanil (92.9%) was administered to 50 male and 50 female Sprague-Dawley Crl:CD®BR VAF/Plus® rats at dose levels of 0 or 2500 ppm (125 mg/kg/day) in the diet. An additional 10 animals/sex were added to each group and sacrificed at 52 weeks. A third group of 15 animals/sex were kept on the study without treatment as sentinel animals. These were also sacrificed at 52 weeks. This study was conducted at the request of the HED Carcinogenicity Peer Review Committee because it was determined that the previous study (MRID No. 00165247) was not conducted at sufficiently high dose levels. It was agreed that the rat carcinogenicity study (not the chronic phase) should be repeated in both sexes with the highest dose approaching the "maximum tolerated dose" level (e.g., about 2500 ppm).

At 2500 ppm, statistically significant increases in liver weight (absolute and/or relative) were observed in treated males (20% and 28% higher) and females (0% and 22% higher) at the interim kill, but not at the terminal kill (4.5% and 8.4% higher (♂) and 8.4% and 4.2% higher (♀). Absolute and relative testes weights were significantly lower in treated males at both the interim (63% and 68% of controls for right testes, 58% and 63% of controls for left testes) and terminal (81% and 85% of controls

for right testes and 73% and 74% of controls for left testes) sacrifices. Increases in the incidences of centrilobular to midzonal hepatocellular enlargement and vacuolization were observed in both sexes. These lesions were observed at both 52 weeks and terminal sacrifice. In the testes, an increase in bilateral aspermatogenesis was observed. The decreased spermatogenic activity was associated with an increase in the incidence of hypospermia and cellular debris in the epididymides of the treated males. In addition, an increased incidence of arteritis/periarteritis was noted in the testes of rats which either died on test or were sacrificed in extremis.

In this study, the NOEL could not be established because there were effects at the only dose level tested (decreases in absolute and relative testes weights; increases in the incidences of centrilobular to midzonal hepatocellular enlargement and vacuolization in the liver of both sexes; increases in bilateral aspermatogenesis in the testes; increases in the incidence of hypospermia and cellular debris in the epididymides; and increased incidence of arteritis/periarteritis in the testes).

Myclobutanil was not oncogenic when tested on Sprague-Dawley rats under the conditions of the study.

The study is classified as Core Guideline when used in conjunction with the previously conducted study (MRID No. 00165247, executive summary below). The two studies together satisfy the regulatory requirement for a chronic feeding/oncogenicity study in the rat (83-5).

In the previously conducted study, technical myclobutanil (90.4% and 91.4% pure) was administered to 110 male and 110 female Sprague-Dawley rats in the diet for a period of 24 months. Dietary levels for the low-dose group were 25/35/50 ppm; for the mid-dose group were 100/140/200 ppm and for the high dose group were 400/560/800 ppm. The first dose level in each series was administered for a period of 2 weeks, the second dose level in each series was administered for a period of 2 weeks and the third dose level in each series was administered from weeks 5 to term. The overall calculated mean daily compound consumption was 0, 2.49, 9.84 or 39.21 mg/kg/day for males and 0, 3.23, 12.86 or 52.34 mg/kg/day for females. Of the 110 animals/dose group, ten/sex/dose group were sacrificed at 3 and 6 months, 20/sex/dose group were sacrificed at 12 months and 18 males and 10 females/dose group were sacrificed at 17 months. A sentinel animal program was also conducted separately in which 30 animals/sex were evaluated at 3, 6 and 12 months.

At 9.84 mg/kg/day in males, the mean absolute testes weights were significantly less than controls ($p \leq 0.05$, 77% of controls) at study termination. In addition, an increase in testicular atrophy was observed. In females (12.86 mg/kg/day),

liver mixed function oxidase activity was significantly increased (61% higher) at 3 months, but not after that time. At 39.21 mg/kg/day (52.34 mg/kg/day in females), a statistically significant increase in liver mixed function oxidase activity was observed (34-47% higher) at 3 and 6 months in males and at 3 months in females (78% higher). In females, relative liver weights were significantly increased at 3 months (13% higher) and absolute liver weights were increased at 6 months (20% higher). Statistically significant decreases in absolute testicular weights were observed in males at 12 months (88%). At termination, decreases in absolute and relative testicular weights were also observed (75% and 79% of controls, respectively). In addition, an increase in testicular atrophy was observed.

The LEL is 9.84 mg/kg/day, based on decreases in testes weights and increases in testicular atrophy. The NOEL is 2.49 mg/kg/day.

The study is classified as Core Guideline when used in conjunction with the present study. The two studies together satisfies the regulatory requirement for a chronic feeding/oncogenicity study in the rat (83-5).

Oncogenicity Studies in the Mouse

In an 18-month carcinogenicity study, technical myclobutanil (92.9%) was administered to 60 female Crl:CD®-1(ICR)BR mice at dose levels of 0 or 2000 ppm (393.5 mg/kg/day) in the diet. Ten from each group were sacrificed at 52 weeks. An additional 20 animals were added to the study as sentinels and also sacrificed at 52 weeks. This study was conducted at the request of the HED Carcinogenicity Peer Review Committee because it was determined that the previous study (MRID No. 00164990) was not conducted at sufficiently high dose levels in females. It was agreed that the mouse carcinogenicity study should be repeated in females with the highest dose approaching the "maximum tolerated dose" level (e.g., about 2000 ppm).

At 2000 ppm, statistically significant decreases in body weight (2 - 12%) and body weight gain (12 - 26%); increases in liver weights (22.9 - 33.2%); hepatocellular hypertrophy; hepatocellular vacuolation; necrosis of single hypertrophied hepatocytes; yellow-brown pigment in the Kupffer cells and cytoplasmic eosinophilia and hypertrophy of the cells of the zona fasciculata area of the adrenal cortex. With the exception of the pigment in the Kuppfer cells, these lesions were present at the 12-month sacrifice.

In this study, the NOEL could not be established because there were effects at the only dose level tested (decreases in body weight and body weight gain; increases in liver weights;

hepatocellular hypertrophy; hepatocellular vacuolation; necrosis of single hypertrophied hepatocytes; yellow-brown pigment in the Kupffer cells and cytoplasmic eosinophilia and hypertrophy of the cells of the zona fasciculata area of the adrenal cortex).

Myclobutanil was not oncogenic when tested on Crl:CD®-1(ICR)BR mice under the conditions of the study. However, it is noted that this study is an 18-month study whereas the old study is a 24-month study.

The study is classified as Core Minimum when used in conjunction with the previously conducted study (MRID No. 00164990, executive summary below). The two studies together satisfy the regulatory requirement for an oncogenicity study in the mouse (83-2).

In the previously conducted study, technical myclobutanil (90.4%) was administered to 110 male and 110 female Crl:CD®-1(ICR)BR mice in the diet for a period of 24 months at the following dose levels: 0, 20, 100 or 500 ppm. The overall calculated mean daily compound consumption was 0, 2.7, 13.7 or 70.2 mg/kg/day for males and 0, 3.2, 16.5 or 85.2 mg/kg/day for females. Of the 110 animals/dose group, ten/sex/dose group were sacrificed at 3 and 6 months and 20/sex/dose group were sacrificed at 12 months. A sentinel animal program was also conducted separately in which 25 animals/sex were evaluated at 24 months.

At 100 ppm, an increase in hepatic mixed-function oxidase (MFO) was observed at 3 months in females (by 29% per mg protein, by 34% per g liver; $p \leq 0.05$). At 500 ppm the following was observed: an increase in MFO in both sexes at 3 (119% (♂) and 61% (♀) above control per total liver) and 6 months (212% (♂) and 210% (♀) above control per total liver) and in females at 12 months (266% above control per total liver); an increase in SGPT values in females at 3 months (59% above controls) and an increase in absolute and relative liver weights in males (10%) and in females (12% and 13%, respectively) at 3 months. In addition, the following treatment-related changes were observed microscopically after 3, 6 and 12 months of treatment in males: increased incidence and severity of centrilobular hepatocytic hypertrophy, Kupffer cell pigmentation, periportal punctate vacuolation and individual hepatocellular necrosis. Following 24 months of treatment, increased incidences of focal hepatocellular alterations and multifocal hepatocellular vacuolation were observed in both sexes. These were not associated with any hypertrophy, hyperplasia or neoplastic proliferations in the liver.

At the time the DER was written, the LEL was considered to be 100 ppm (16.5 mg/kg/day) based on an increase in hepatic mixed-function oxidase (MFO) at 3 months in females. The NOEL

was 20 ppm (3.2 mg/kg/day). The increase in MFO at 3 months in females is not considered to be a significant enough effect to establish an LEL. Therefore, the LEL is raised to 500 ppm (70.2 mg/kg/day for males and 85.2 mg/kg/day for females) based on increases in MFO in both sexes; increases in SGPT values in females and in absolute and relative liver weights in both sexes at 3 months; increased incidences and severity of centrilobular hepatocytic hypertrophy, Kupffer cell pigmentation, periportal punctate vacuolation and individual hepatocellular necrosis in males; and increased incidences of focal hepatocellular alterations and multifocal hepatocellular vacuolation in both sexes. The NOEL is 100 ppm (16.5 mg/kg/day).

The study is classified as Core Minimum when used in conjunction with the present study. The two studies together satisfies the regulatory requirement for an oncogenicity study in the mouse (83-2).

Chronic Feeding Study in the Dog

Technical myclobutanil (91.4%) was fed in the diet to 6 male and 6 female Beagle dogs per group at dose levels of 0, 10, 100, 400 or 1600 ppm for one year.

At 400 ppm, hepatocellular hypertrophy was observed in both sexes. Increases in liver weights (131% absolute, 128% relative) were observed in females. At 1600 ppm, hepatocellular hypertrophy in both sexes and "ballooned" hepatocytes, centrilobular were observed in females. These were supported by increases in liver weights (130% absolute, 144% relative in males; 154% absolute, 152% relative in females); increases in alkaline phosphatase (160 - 243% in males; 296 - 381% in females) increases in SGPT in males ((124 - 137%) and increases in GGT in females (200%). In addition, slight changes in hematological parameters were observed in males (decreases in RBC, 89.6% - 90.7%; increases in platelets, 125.3 - 161.3%; slight increases in MCH, 104 - 106%; and MCV, 102 - 103%). There were also a slight increases in phosphorus in males (114 - 133%) and a slight decrease in albumin (88 - 91% in males and 91-94% in females). **The LEL is 400 ppm (14.28 mg/kg/day) and the NOEL is 100 ppm (3.09 mg/kg/day) based on hepatocellular hypertrophy, increases in liver weights, "ballooned" hepatocytes and increases in alkaline phosphatase, SGPT and GGT. In addition, there were some possible slight hematological effects.**

The study is Core-Minimum because full histopathology examinations were not submitted for the mid- and low dose level groups. It satisfies the requirement for 83-1(b) chronic feeding study in the dog.

Developmental Toxicity Study in the Rat

In a developmental toxicity study, 25 Sprague-Dawley [Crl:CD-(SD)BR] rats/dose group received either 0, 31.26, 93.77, 312.58 or 468.87 mg/kg/day technical myclobutanil (84.5% a.i.) by oral gavage from gestation day 6 through 15, inclusive. The animals received the test material in a dose volume of 10 ml/kg. The females were mated to proven males on a one-to one basis for up to 5 days. The day on which sperm were noted was designated as gestation day 0.

Maternal toxicity was not observed at either 31.3 or 93.8 mg/kg/day. At 312.6 mg/kg/day, clinical signs of toxicity consisting of rough hair coat and salivation were observed. At 468.87 mg/kg/day, rough hair coat, salivation, alopecia, desquamation and red exudate around the mouth were observed. No other effects were observed. **The maternal toxicity LOEL is 312.6 mg/kg/day and the maternal toxicity NOEL is 93.8 mg/kg/day based on clinical signs of toxicity.**

A significant increase in the incidences of 14th rudimentary ribs and 7th cervical ribs was observed at 312.6 and 468.9 mg/kg/day. No other developmental effects were observed at any dose level tested. **The developmental toxicity LOEL is 312.6 mg/kg/day and the developmental toxicity NOEL is 93.8 mg/kg/day based on increased incidences of 14th rudimentary and 7th cervical ribs.**

The study is classified as Core Minimum Data (Acceptable) and satisfies the requirement (83-3 a) for a developmental toxicity study in rats.

Developmental Toxicity Study in the Rabbit

RH-53,866 (technical myclobutanil, 90.4%) was tested in a developmental toxicity study in rabbits. Five groups of 18 female New Zealand White rabbits from Hazleton Dutchland, Denver, PA, received 0 (water control), 0 (Hi-Sil control), 20.0, 60.0 or 200.0 mg/kg/day a.i. mycobutanil by oral gavage (5 ml/kg b.w.) on days 7 through 19 of gestation. The test material was prepared with the solid adsorbent (carrier) Hi-Sil 233 and with 1.0% methylcellulose (vehicle). The females were injected i.v. with chorionic gonadotropin and artificially inseminated 3 hours later. The day on which insemination occurred was considered to be day 0 of gestation.

No maternally toxic effects were observed at either 20 or 60 mg/kg/day. At 200 mg/kg/day, decreases in maternal body weight and body weight gain during the dosing period (0.03 and -0.02 kg in the controls versus -0.28 kg in the treated group) with a rebound during the post-dosing period, irregular feces,

bloody urine, bloody urogenital or anal area and blood and/or aborted material were observed in the drop pan. Three does aborted their litters, however, 2 of those litters were totally resorbed (early). There were no effects noted at necropsy. **The maternal toxicity LOEL = 200 mg/kg/day and the maternal toxicity NOEL = 60 mg/kg/day based on reduced body weight and body weight gain during the dosing period, clinical signs of toxicity and possibly abortions.**

No developmentally toxic effects were observed at either 20 or 60 mg/kg/day. At 200 mg/kg/day, there were increases in resorptions, resorptions/litter, decreases in litter size ($p < 0.05$) and a decrease in the viability index ($p < 0.05$). **The LOEL for developmental toxicity is 200 mg/kg/day and the NOEL for developmental toxicity is 60 mg/kg/day based on increases in resorptions, decreases in litter size and a decrease in the viability index.**

The study is classified as Core Minimum data (acceptable) and satisfies the requirement (83-3 b) for a developmental toxicity study in rabbits.

2-Generation Reproduction Study in the Rat

RH-53,866 (technical myclobutanil, 84.5% pure) was tested in a 2-generation reproduction study with male and female CRL:CD(SD)BR rats. The rats were obtained from Charles River Breeding Laboratories, Kingston Facility, Stone Ridge, NY. Twenty-five animals/sex/dose group received 0, 50, 200 or 1000 ppm in the diet throughout the study (0, 2.5, 10 or 50 mg/kg/day by standard conversion factor). The animals were mated on a one to one ratio with the F_0 parental animals and were given test diets for 8 weeks before they were mated. Selection of the parents for the F_1 generation was made when the pups were 25 days of age, and the mated animals in the study were approximately 81 days of age at mating.

At 200 ppm, centrilobular hepatocellular hypertrophy was observed in the P_2 males. This was supported by slight but statistically significant increases in liver weights in males: (114% absolute, 107% relative for P_1 and 107% absolute and 104% relative for P_2). At 1000 ppm, centrilobular hepatocellular hypertrophy was observed in both sexes in the P_1 and P_2 generations. These were again supported by slight but statistically significant increases in liver weights: males: (113.6% absolute, 114% relative for P_1; 107% absolute, 113% relative for P_2); females: (109% absolute, 109% relative for P_1; 106% absolute, 108% relative for P_2). Therefore, **the parental (systemic) toxicity LOEL is 200 ppm and the parental (systemic) toxicity NOEL is 50 ppm** based on hepatocellular hypertrophy and increases in liver weights.

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At 1000 ppm, an increase in the number of stillborn or % born dead was observed in both generations (4.9 - 5.3% versus 0 - 1.9% in controls). In addition, multifocal or diffuse testicular atrophy was observed in males in the P_2 generation. Increased necrotic spermatocytes/spermatids or decreased spermatozoa and atrophy of the prostate were also observed in these animals. Therefore, **the reproductive toxicity LOEL is 1000 ppm and the reproductive toxicity NOEL is 200 ppm** based on an increased incidence in the number of stillborns and atrophy of the testes and prostate.

At 1000 ppm, it appears that there was a decrease in pup weight gain during lactation (83.3% to 89.7% of the controls). Therefore, the **developmental toxicity LOEL is 1000 ppm and the developmental toxicity NOEL is 200 ppm** based on a decrease in pup body weight gain during lactation.

This study is classified as Core Guideline and satisfies the regulatory requirement for a multigeneration reproduction study in the rat (83-4).

Toxicology Profile

Last Updated: 5/26/94

Technical grade Myclobutanil (Rally)

Guide-line #	Study Identification and Classification	Results
81-1	Acute Oral Toxicity in Rats MRID 072896 Report # 84-063A Date: 7/19/84 Acceptable	LD_{50}: 1.6 g/kg (males) LD_{50}: 2.29 g/kg (females) TOXICITY CATEGORY: III
81-2	Acute Dermal Toxicity in Rabbits MRID 072896 Report # 84R-134A Date: 7/30/84 Acceptable	LD_{50}: >5000 mg/kg TOXICITY CATEGORY: III
81-3	Acute Inhalation Toxicity in Rats MRID 403571-01 Report # 87R-028 Date: 8/31/87 Acceptable	LC_{50}: >5.1 mg/L (Four hour exposure) TOXICITY CATEGORY: IV
81-4	Primary Eye Irritation in Rabbits MRID 072896 Report # 84R134A Date: 8/3/84 Acceptable	Primary Irritation Score: Not given in DER. TOXICITY CATEGORY: I Severe eye irritant
81-5	Primary Dermal Irritation in Rabbits MRID 072896 Report # 84R-134A Date: 8/3/84 Acceptable	Primary Irritation Score: Not given in DER TOXICITY CATEGORY: IV Non-irritating to the skin under conditions of test.

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Last Updated: 5/26/94

Technical grade Myclobutanil (Rally)

Guide-line #	Study Identification and Classification	Results
81-6	Dermal Sensitization in Guinea Pigs MRID 403571-02 Report # 87R-035 Date: 6/25/87 Acceptable	Buehler method. 12 pigs, 10 induction doses of 0.4 ml of 50% w/w formulation in 80% ethanol. 50% w/w formulation in acetone used at challenge + rechallenge. Minimal erythema at 24 & 48 hrs. Positive sensitizing reaction.
82-1 (a)	Subchronic Feeding in Rats (13 weeks) MRID 072897-98 Report # 83R-068 Date: 8/7/84 Core Grade: Minimum	NOEL: 1000 ppm LOEL: 3000 ppm Effects: increased liver, kidney wts.; hypertrophy, necrosis in liver; pigmentation in convoluted kidney tubules; vacuolated adrenal cortex.
82-1 (b)	Subchronic Feeding in Dogs (13 Weeks) MRID 072899-900 Report # 83R-204 Date: 8/7/84 Core Grade: Minimum	NOEL: 10 ppm LOEL: 200 ppm Effects: Centrilobular or midzonal hepatocellular hypertrophy.
83-1	Chronic feeding study in dogs MRID 00165248 Report # 84R-078 Date: 10/15/86 Core Grade: Minimum	NOEL: 100 ppm LOEL: 400 ppm Effects: 91.4% material fed in the diet to 6 dogs/group/dose at levels of 0, 10, 100, 400 or 1600 ppm for one year. LEL: 14.28 mg/kg/day; the NOEL: 3.09 mg/kg/day based on hepatocellular hypertrophy, increases in liver weights, "ballooned" hepatocytes and increases in alkaline phosphatase, SGPT and GGT. In addition, there were some possible slight hematological effects. Full histopathology examinations not submitted for mid- and low dose level groups.

Last Updated: 5/26/94

Technical grade Myclobutanil (Rally)

Guide-line #	Study Identification and Classification	Results
83-2 (a)	Oncogenicity study in mice MRID 00164990 Report # 84R-023 Date: 10/17/86 Core Grade: Minimum when considered with MRID 428091-02	NOEL: 100 ppm (Systemic) LOEL: 500 ppm (Systemic) Effects: 90.4% test material given to male & female Crl:CD®-1(ICR)BR mice in diet for 24 months at 0, 20, 100 or 500 ppm (0, 2.7, 13.7 or 70.2 mg/kg/day ♂; 0, 3.2, 16.5 or 85.2 mg/kg/day ♀). LEL based on ↑ MFO (♂+♀); ↑ SGPT (♀) & ↑ absolute & relative liver wts (♂+♀); ↑ incidences and severity of centrilobular hepatocytic hypertrophy, Kupffer cell pigmentation, periportal punctate vacuolation & individual hepatocellular necrosis (♂); & ↑ incidences of focal hepatocellular alterations and multifocal hepatocellular vacuolation (♂+♀). Not tested at high enough dose levels in females. MRID No. 428091-02 tested at sufficiently high dose levels (2000 ppm (393.5 mg/kg/day)), no oncogenic effects observed. The two studies together satisfy the regulatory requirement for an oncogenicity study in the mouse.

Last Updated: 5/26/94

Technical grade Myclobutanil (Rally)

Guide-line #	Study Identification and Classification	Results
83-2 (a)	Oncogenicity study in mice MRID 428091-02 Report # 89R-261 Date: 3/17/93 Core Grade: Minimum when considered with MRID 00164990	NOEL: Not established LOEL: 2000 ppm (393.5 mg/kg/day) Effects: Technical (92.9%) administered to female Crl:CD®-1(ICR)BR mice at 0 or 2000 ppm (393.5 mg/kg/day) in diet. Decreases in body wt & body wt gain; increases in liver wts; hepatocellular hypertrophy; hepatocellular vacuolation; necrosis of single hypertrophied hepatocytes; yellow-brown pigment in the Kupffer cells and cytoplasmic eosinophilia and hypertrophy of the cells of the zona fasciculata area of the adrenal cortex. Not oncogenic under the conditions of the study. Study is only 18 months, however, the two studies together satisfy the regulatory requirement for an oncogenicity study in the mouse.

Last Updated: 5/26/94

Technical grade Myclobutanil (Rally)

Guide-line #	Study Identification and Classification	Results
83-2 (b)	Oncogenicity study in rats MRID 428091-01 Report # HWA 417-471, RH-89RC-260 Date: 2/12/93 Core Grade: Guideline when taken in conjunction with MRID 00165247	NOEL: Not established LOEL: 2500 ppm (only dose tested) Effects: (92.9%) administered to ♂+♀ Sprague-Dawley Crl:CD®BR VAF/Plus® rats at 0 or 2500 ppm (125 mg/kg/day) in the diet. Testicular atrophy and decreases in testes weights; increases in the incidences of centrilobular to midzonal hepatocellular enlargement and vacuolization in the liver of both sexes; increases in bilateral aspermatogenesis in the testes; increases in the incidence of hypospermia and cellular debris in the epididymides; and increased incidence of arteritis/periarteritis in the testes). No oncogenic effects observed. Satisfies regulatory requirement when taken with MRID 00165247.
83-3	Teratology Study in Rabbits MRID 00164971 Report # 83R-217 Date: 11/15/84 Core Grade Minimum	Maternal NOEL: 60 mg/kg/day Maternal LOEL: 200 mg/kg/day Effects: technical 90.4% administered to ♀ New Zealand White rabbits 0 (water control), 0 (Hi-Sil control), 20.0, 60.0 or 200.0 mg/kg/day a.i. by oral gavage (5 ml/kg b.w.) on days 7 - 19 of gestation. Reduced body weight and body weight gain during the dosing period, clinical signs of toxicity and possibly abortions. Developmental NOEL: 60 mg/kg/day Developmental LOEL: 200 mg/kg/day Effects: Increases in number of resorptions, decreases in litter size and a decrease in the viability index.

Toxicology Profile

Last Updated: 5/26/94

Technical grade Myclobutanil (Rally)

Guide-line #	Study Identification and Classification	Results
83-3	Teratology Study in Rats MRID 00141672 Report # 83R-024 Date: 6/22/84 Core Grade Minimum	Maternal NOEL: 93.8 mg/kg/day Maternal LOEL: 312.6 mg/kg/day Effects: Technical (84.5 %) administered to Sprague-Dawley [Crl:CD-(SD)BR] rats at 0, 31.26, 93.77, 312.58 or 468.87 mg/kg/day by oral gavage from gestation days 6 - 15, inclusive. Rough hair coat and salivation at 312.6 and salivation, alopecia, desquamation and red exudate around mouth at 468.87 mg/kg/day. Developmental NOEL: 93.8 mg/kg/day Developmental LOEL: 312.6 mg/kg/day Effects: Increased incidences of 14th rudimentary and 7th cervical ribs at 312.6 and 468.9 mg/kg/day.

Last Updated: 5/26/94

Technical grade Myclobutanil (Rally)

Guide-line #	Study Identification and Classification	Results
83-4	Multigeneration Reproduction Toxicity in Rats MRID 00143766 and 00149581 Report # 84R-117 Date: 8/21/85 Core Grade Guideline	Systemic NOEL: 50 ppm (2.5 mg/kg/day) Systemic LOEL: 200 ppm (10 mg/kg/day) Effects: Technical (84.5% pure) administered to male and female CRL:CD(SD)BR rats at 0, 50, 200 or 1000 ppm in diet (0, 2.5, 10 or 50 mg/kg/day). ↑ liver weights and hepatocellular hypertrophy. Reproductive NOEL: 200 ppm (10 mg/kg/day) Reproductive LOEL: 1000 ppm (50 mg/kg/day) Effects: Increased incidence in the number of stillborns and atrophy of the testes, epididymides and prostate. Developmental NOEL: 200 ppm (10 mg/kg/day) Developmental LOEL: 1000 ppm (50 mg/kg/day) Effects: Decrease in pup body weight gain during lactation.

Last Updated: 5/26/94

Technical grade Myclobutanil (Rally)

Guide-line #	Study Identification and Classification	Results
83-5	Chronic Feeding/ Oncogenicity study in rats MRID 00165247 Report # 85RC-61 Date: 10/24/86 Core Grade: Guideline when taken in conjunction with MRID 428091-01	NOEL: 2.49 mg/kg/day LOEL: 9.84 mg/kg/day Effects: Technical (90.4% and 91.4% pure) administered to ♂+♀ Sprague-Dawley rats in diet for 24 months at 25/35/50, 100/140/200 & 400/560/800 ppm (2 weeks/2 weeks/to termination; 0, 2.49, 9.84 or 39.21 mg/kg/day (♂); 0, 3.23, 12.86 or 52.34 mg/kg/day (♀). ↓ testes wts & ↑ in testicular atrophy. Not tested at high enough dose levels. MRID No. 428091-01 tested at sufficiently high dose levels (2500 ppm: 125 mg/kg/day), no oncogenic effects observed. Satisfies regulatory requirment when taken with MRID 428091-01.
84-2 (a)	Gene Mutation Assay (Ames Test) MRID 072901 Report # 83R-0246 Date: 1/31/84 Acceptable	No appreciable increase in the reversion to histidine protrophy of 4 S. typhimurium strains at 75 to 7500 ug/plate with & without S-9 activation.
84-2 (a)	Gene Mutation Assay Mammalian Cells MRID 072901 Report # 84R-046 Date: 5/29/84 Acceptable	Negative with and without metabolic activation up to 175 ug/ml.

Last Updated: 5/26/94

Technical grade Myclobutanil (Rally)

Guide-line #	Study Identification and Classification	Results
84-2 (b)	Structural Chromosomal Aberration Assay In vivo cytogenetics MRID 072901 Report # 84R-0074 Date: 7/23/84 Acceptable	The level of 650 mg/kg did not cause a significant increase in chromosomal aberrations in bone marrow cells sampled over the entire mitotic cycle.
84-2 (b)	Structural Chromosomal Aberration Assay In vitro cytogenetics MRID 266099 Report # 20990 Date: 4/85 Acceptable	Did not induce chromosomal aberrations with & without metabolic activation under the conditions of the study up to 200 ug/ml.
84-2 (b)	Structural Chromosomal Aberration Assay Dominant Lethal Test MRID 266101 Report # 86RC-0054 Date: 10/10/86 Acceptable	Did not induce dominant lethal mutations under conditions of study at dose levels up to 735 mg/kg.
84-2 (c)	Other Genotoxicity Assays (Unscheduled DNA Synthesis) MRID 266100 Report # 86R-084 Date: 7/22/86 Acceptable	Did not induce an increase in unscheduled DNA synthesis up to toxic dose. 0.1-1000 ug/ml tested.

Last Updated: 5/26/94

Technical grade Myclobutanil (Rally)

Guide-line #	Study Identification and Classification	Results
85-1	Metabolism MRID 266102 Report # 83R-175 Date: 8/29/86 Acceptable	Rapidly absorbed and excreted. Completely eliminated by 96 hrs. Extensively metabolized prior to excretion. Metabolic patterns similar for both sexes. Disposition & metabolism after pulse administration is linear over dose range.
85-1	Metabolism MRID 266103 Report # 83R-144 Date: 8/28/86 Acceptable	Completely and rapidly absorbed. Extensively metabolized and rapidly and essentially completely excreted. Elimination of label from plasma biphasic and evenly distrib. between urine and feces. No tissue accumulation after 96 hours.
85-1	Metabolism MRID 072904 Report # 310-84-16 Date: 6/22/84 Acceptable	At least 7 major metabolites recovered and identified. Highest amounts of radioactivity found in liver, kidneys, large and small intestines. No tissue accumulation.

Toxicology Profile

Last Updated: 11/07/90

% Formulation 40%

Guide-line #	Study Identification and Classification	Results
81-1	Acute Oral Toxicity in Rats MRID 072896 Report # 84R-082A & 84R-082B Date: 7/16/84 Acceptable	LD_{50}: 1.87 g/kg (males) LD_{50}: 2.09 g/kg (females) TOXICITY CATEGORY: III
81-2	Acute Dermal Toxicity in Rabbits MRID 072896 Report # 84R-082A & 84R-082B Date: 7/16/84 Acceptable	LD_{50}: >5 g/kg TOXICITY CATEGORY: III
81-3	Acute Inhalation Toxicity in Rats MRID 072896 Report # 84R-047 Date: 6/27/84 Acceptable	LC_{50}: >5.0 mg/L (Four hour exposure) TOXICITY CATEGORY: IV
81-4	Primary Eye Irritation in Rabbits MRID 266026 Report # 86R-0193 Date: 10/21/86 Acceptable	Primary Irritation Score: 17.1 TOXICITY CATEGORY: II Moderately irritating to the eye. Irritation cleared in 8-21 days.
81-5	Primary Dermal Irritation in Rabbits MRID 072896 Report # 84R-082A Date: 7/16/84 Acceptable	Primary Irritation Score: Not given TOXICITY CATEGORY: IV Mild irritant.

Last Updated: 11/07/90

% Formulation 40%

Guide-line #	Study Identification and Classification	Results
81-6	Dermal Sensitization in Guinea Pigs MRID 403571-03 Report # HLA 417-424 Date: 8/26/87 Acceptable	Not a sensitizer. 19 pigs received 10 doses of 0.4 ml doses of 50% w/w test chemical. Challenge of 0.1% DCNB in acetone. Positive control DCNB.

Last Updated: 11/07/90

% Formulation 40%

Guide-line #	Study Identification and Classification	Results
82-2	28-day dermal in rats Accession No. 266080 Report # 85R-240 Date: 8/29/86 Minimum	NOEL for systemic effects: > 100 mg a.i./kg/day. NOEL for skin irritation: 10 mg a.i./kg/day LEL: 100 mg a.i./kg/day for both formulations. Effects: Study conducted on two formulations: 41.36% (40WP) and 24.99% (2EC) formulation. 2EC formulation applied at either 1, 10 or 100 mg a.i./kg and 40WP formulation applied at 100 mg a.i./kg. Rats treated 1x/day for a total of 19-20 treatments over a 4-week period. No systemic effects observed at any dose level for either formulation. Microscopic changes, indicating irritation were observed in the skin. These included epidermal necrosis, epidermal thickening, and/or subacute/chronic inflammation of the dermis and were observed in all groups, including controls, however, the changes were of lesser severity and at a lower incidence in the vehicle control and in the mid- and low dose groups of the 2EC formulation. The 40WP group exhibited a minimal to mild degree of chronic inflammation and epidermal thickening with 2 animals exhibiting eschar formation. This study is acceptable for regulatory requirement for a 21-day dermal study for both the technical and the formulation (see Data Gaps Comments).

Data Gaps: The following toxicity studies are recommended to be submitted in support of registration and tolerances for technical myclobutanil (Rally). Those recommendations that have been satisfied are indicated:

	Required	Satisfied
Acute oral toxicity	Yes	Yes
Acute dermal toxicity	Yes	Yes
Acute inhalation	Yes	Yes
Primary eye irritation	Yes	Yes
Primary dermal irritation	Yes	Yes
Dermal sensitization	Yes	Yes
90-day feeding		
rodent	Yes	Yes
nonrodent	Yes	Yes
21-day dermal	Yes	Yes[1]
Chronic feeding		
rodent	Yes	Yes
nonrodent	Yes	Yes
Oncogenicity		
rat	Yes	Yes[2]
mouse	Yes	Yes[2]
Teratology		
rat	Yes	Yes
rabbit	Yes	Yes
Reproduction	Yes	Yes
Gene mutation	Yes	Yes
Chromosomal aberration	Yes	Yes
Other genotoxic effects	Yes	Yes
Metabolism	Yes	Yes

Comments

1. In a memorandum dated February 12, 1986 from J.E. Harris to H. Jacoby, Dr. Harris stated that a 21-day dermal study on RH-3866 should be conducted with particular attention given to any possible testicular effects. Since acceptable 4-week dermal studies on Rally™ 40WP and on another RH-3866 formulation (2EC) have been evaluated and found to be negative for testicular effects, the requirement for a 21-day dermal study is considered by TB-I to have been satisfied.

2. A previous conditional registration and tolerance for myclobutanil on apples and grapes has been granted to the Registrant on the condition that the rat oncogenicity study be repeated in both sexes and that the mouse oncogenicity

study be repeated in females. In both cases, the MTD was not reached. These studies have been repeated at the dose level(s) requested. When combined with the previous studies, the new studies satisfy the regulatory requirements for an oncogenicity study in mice and a chronic feeding/oncogenicity study in rats.

Actions Being Taken to Obtain Additional Information or Clarification:
None.

Reference Dose (RfD):

The recommended RfD (to the RfD Workgroup) is 0.025 mg/kg/day. This value was calculated by using the Chronic Rat Feeding Study NOEL of 2.49 mg/kg/day and a safety factor of 100. This RfD has been verified or approved by the Health Effects Division and the Agency RfD Committees.

Pending Regulatory Actions: None.

Toxicologic Issues Pertinent to This Request: None.

Reviewed By: Pamela Hurley, Toxicologist Pamela M. Hurley 4/12/94
Section I, Tox. Branch (7509C)
Secondary Reviewer: Roger L. Gardner, Section Head
Section I, Tox. Branch (7509C) Roger Gardner 4/13/94

DATA EVALUATION RECORD

011124

STUDY TYPE: Chronic dog (83-1(b)) - **Supplemental DER**

SHAUGHNESSY NO./TOX. CHEM. NO.: 128857 / 723K

ACCESSION NO./MRID NO.: 266088 / 00165248

TEST MATERIAL: RH-3866

SYNONYMS: Myclobutanil, Rally, Systhane, Nova

REPORT NUMBER: 84R-078

SPONSOR: Rohm and Haas Co., Philadelphia, PA

TESTING FACILITY: Rohm and Haas Co., Toxicology Dept., Spring House, PA

TITLE OF REPORT: RH-3866: One Year Dietary Study in Beagle Dogs

AUTHOR(S): P.R. Goldman and J.C. Harris

REPORT ISSUED: October 15, 1986

CONCLUSION: Technical myclobutanil (91.4%) was fed in the diet to 6 male and 6 female Beagle dogs per group at dose levels of 0, 10, 100, 400 or 1600 ppm for one year.

At 400 ppm, hepatocellular hypertrophy was observed in both sexes. Increases in liver weights (131% absolute, 128% relative) were observed in females. At 1600 ppm, hepatocellular hypertrophy in both sexes and "ballooned" hepatocytes, centrilobular were observed in females. These were supported by increases in liver weights (130% absolute, 144% relative in males; 154% absolute, 152% relative in females); increases in alkaline phosphatase (160 - 243% in males; 296 - 381% in females) increases in SGPT in males ((124 - 137%) and increases in GGT in females (200%). In addition, slight changes in hematological parameters were observed in males (decreases in RBC, 89.6% - 90.7%; increases in platelets, 125.3 - 161.3%; slight increases in MCH, 104 - 106%; and MCV, 102 - 103%). There were also a slight increases in phosphorus in males (114 - 133%) and a slight decrease in albumin (88 - 91% in males and 91-94% in females). **The LEL is 400 ppm (14.28 mg/kg/day) and the NOEL is 100 ppm (3.09 mg/kg/day) based on hepatocellular hypertrophy, increases in liver weights, "ballooned" hepatocytes and increases in**

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alkaline phosphatase, SGPT and GGT. In addition, there were some possible slight hematological effects.

The study is Core-Minimum because full histopathology examinations were not submitted for the mid- and low dose level groups. It satisfies the requirement for 83-1(b) chronic feeding study in the dog.

This is a supplemental DER which contains the extra tables needed for a complete analysis.

A. RESULTS:

1. Body Weight Determinations:

Body Weights (g) - Males

Dose (ppm) / Day	0	10	100	400	1600
0	8201.5	8112.2	8152.0	7813.0	8086.2
7	8509.5	8385.3	8294.2	7983.3	8149.2* (96%)
14	8640.0	8428.0	8482.5	8060.3	8279.8
21	8751.2	8506.0	8616.0	8124.5	8344.7
28	8909.8	8504.3	8748.5	8070.2*	8412.7
56	9110.8	8595.5	9206.3	8132.2	8562.0
105	9417.3	8963.0	9675.3	8326.5	8830.0
154	9784.7	8935.8	9936.5	8413.5	8878.5
203	9957.2	9149.5	10064.2	8784.3	9200.2
252	10074.7	9126.3	10201.7	8722.0	9334.7
315	10133.3	9155.3	10074.0	8750.8	9325.8
364	10373.2	8951.5*	9898.8	8976.7	9445.2

* $p < 0.05$

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Body Weights (g) - Females

Dose (ppm) Day	0	10	100	400	1600
0	6637.7	6851.5	6760.3	6707.8	6717.5
7	6893.0	7000.5	6985.5	6802.3	6609.0* (96%)
14	7087.2	7071.0	7115.0	6891.0* (97.2%)	6616.7* (93.4%)
21	7173.5	7164.3	7245.0	6995.3	6717.7* (94.3%)
28	7265.8	7126.3	7322.5	7090.2	6797.3* (93.6%)
56	7514.5	7269.0	7617.2	7263.0	7171.8
105	7592.2	7466.0	7669.3	7513.0	7517.3
154	7612.5	7282.8	7594.2	7662.2	7694.2
203	7565.5	7532.5	8124.0	7843.3	7845.0
252	7825.5	7517.2	8229.0	7935.7	7842.7
315	7938.3	7593.7	8447.7	7967.8	8110.2
364	10373.2	8951.5*	9898.8	8975.7	9445.2

* $p < 0.05$

2. Food and/or Water Consumption:

Food Consumption - Males

Dose (ppm) Days	0	10	100	400	1600
0-6	2100	2040	2070	2049	1898
7-13	2100	2004	2087	2058	2026
21-27	2100	2035	2100	2093	2077
42-48	2087	2073	2100	2088	2100
70-76	2100	2100	2100	2100	2074

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Food Consumption - Males

Dose (ppm) Days	0	10	100	400	1600
119-125	2089	2091	2100	2100	2100
154-160	2100	2094	2100	2100	2100
210-216	2100	2100	2100	2100	2100
259-265	2100	2100	2095	2100	2100
357-363	2100	2100	2100	2100	2100

Food Consumption - Females

Dose (ppm) Days	0	10	100	400	1600
0-6	2044	2031	2033	1857	1269* (62%)
7-13	2046	2092	2097	1993	1887* (92%)
21-27	2084	2088	2098	2054	1897* (91%)
42-48	2050	2100	2100	2073	2006
70-76	2064	2100	2100	2084	1731* (84%)
119-125	2100	2100	2100	2100	1961* (93%)
154-160	1985	2100	2100	2100	1980
210-216	1995	2100	2100	2100	1901* (95%)
259-265	2021	2094	2100	2100	1919* (95%)
357-363	2050	2082	2100	2046	1926* (94%)

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3. Hematology:

Hematology - Males

Dose Parameter	0	10	100	400	1600
RBC $10^6/mm^3$					
Week 13	6.837	6.983	6.500	6.743	6.342
Week 25	6.837	6.812	6.770	6.560	6.198* (90.7%)
Week 39	7.175	6.803	6.997	6.902	6.508* (90.7%)
Week 53	7.572	6.967*	7.355	7.222	6.782* (89.6%)
Plat $10^3/mm^3$					
Week 13	245.7	284.5	266.5	334.3*	396.3* (161.3%)
Week 25	272.5	309.8	259.2	330.2	374.2* (137.3%)
Week 39	326.5	347.7	301.0	366.7	409.2* (125.3%)
Week 53	286.8	371.3*	291.8	345.7	402.3* (140.2%)
MCH ($\mu\mu g$)					
Week 13	21.62	21.65	22.20	21.83	22.52* (104%)
Week 25	22.72	22.93	23.28	23.12	23.75* (105%)
Week 39	22.00	22.48	22.67	22.47* (102%)	23.22* (106%)
Week 53	21.48	22.00	22.25	21.97* (102%)	22.77* (106%)
MCV μ^3					
Week 13	74.0	74.8	75.5* (102%)	74.7	75.8* (102%)
Week 25	74.0	75.2	75.8* (102%)	74.8	76.2* (103%)
Week 39	73.3	74.3	74.8* (102%)	74.0	75.7* (103%)
Week 53	70.8	71.2	72.0* (102%)	71.3	73.0* (103%)

RBC = red blood cells; Plat = platelets; MCH = mean cell hemoglobin; MCV = mean corpuscular volume

4. Clinical Chemistry:

Dose (ppm) Parameter	0	10	100	400	1600
			Clinical Chemistry		
			Males		
P					
Week 13	4.50	4.73	4.65	4.77	5.15* (114%)
Week 25	4.22	4.30	4.30	4.68* (111%)	4.92* (117%)
Week 39	3.38	3.55	3.68	3.70	4.37* (129%)
Week 53	3.25	3.45	4.13*	3.70	4.33* (133%)
AP					
Week 13	68.5	73.2	59.3	78.2	109.3* (160%)
Week 25	55.5	60.5	41.8	66.0	104.5* (188%)
Week 39	43.5	49.8	29.0	53.7	90.8* (208%)
Week 53	40.0	53.2	27.7	53.2	97.3* (243%)
Alb					
Week 13	3.10	3.15	3.08	3.00	2.78* (90%)
Week 25	3.08	3.05	3.02	2.95	2.70* (88%)
Week 39	3.25	3.12	3.22	3.03* (93%)	2.95* (91%)
Week 53	3.27	3.15	3.25	3.08	2.97* (91%)
SGPT					
Week 13	19.8	22.7	20.2	21.5	22.0
Week 25	22.3	23.2	23.2	18.2	30.5* (137%)
Week 39	21.0	21.0	20.2	16.7	25.7
Week 53	23.7	23.2	23.2	20.5	29.3* (124%)

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Clinical Chemistry

Dose (ppm) Parameter	0	10	100	400	1600
			Females		
P					
Week 13	4.40	4.48	4.57	4.63	4.57
Week 25	4.07	4.40	4.23	4.15	4.50
Week 39	3.43	3.25	3.40	3.33	3.50
Week 53	3.42	3.70	3.92	3.78	3.90* (114%)
AP					
Week 13	72.2	89.0	102.2	102.2	213.7* (296%)
Week 25	68.5	73.2	81.2	101.3	211.0* (308%)
Week 39	57.2	57.5	58.8	90.5	218.2* (381%)
Week 53	57.5	60.7	59.0	91.7* (159%)	187.0* (325%)
Alb					
Week 13	3.23	3.30	3.18	3.23	2.93* (91%)
Week 25	3.80	4.07	4.13	3.82	3.77
Week 39	3.12	3.33* (107%)	3.28	3.20	2.93* (94%)
Week 53	3.28	3.38	3.42	3.30	3.13
GGT					
Week 13	0.7	1.3	1.0	1.7	1.3
Week 25	1.7	2.5	2.2	2.2	3.2
Week 39	0.7	0.3	0.4	0.7	2.2
Week 53	2.0	2.0	1.6	1.8	4.0* (200%)

P = Inorganic phosphorus (mg/deciliter); AP = alkaline phosphatase (Units/Liter); Alb = serum albumin (g/deciliter), SGPT = serum glutamic-pyruvic transaminase (Units/Liter), GGT = gamma glutamyl transferase (units/liter)
* $p < 0.05$; ** $p < 0.01$

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5. Organ Weights:

Liver Weights at Termination (g)

	Male		Female	
Dose (ppm)	Absolute	Relative	Absolute	Relative
0	299	295	226	290
10	265	300	260	346* (119%)
100	291	294	281* (124%)	330
400	291	337	295* (131%)	370* (128%)
1600	389* (130%)	424* (144%)	349* (154%)	441* (152%)

* $p < 0.05$
Relative = Organ weight x 10,000/ Body weight

B. DISCUSSION:

This study is a core minimum study because full histopathology examinations were not submitted for the mid- and low dose level groups. However, it does satisfy the requirement for 83-1(b) chronic feeding study in the dog. The LEL is 400 ppm (14.28 mg/kg/day) and the NOEL is 100 ppm (3.09 mg/kg/day) based on hepatocellular hypertrophy, increases in liver weights, "ballooned" hepatocytes and increases in alkaline phosphatase, SGPT and GGT. In addition, there were some possible slight hematological effects.

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Reviewed by: Pamela Hurley
Section 2 , Tox. Branch (TS-769C)
Secondary Reviewer: Edwin Budd
Section 2 , Tox. Branch (TS-769C)

Budd 11/2/88

DATA EVALUATION REPORT

STUDY TYPE: Chronic Feeding Nonrodent (Dog) (83-1)

TOX. CHEM. NO.: 723K

ACCESSION NUMBER: 266088

TEST MATERIAL: RH-3866

SYNONYMS: Systhane, Rally, Myclobutanil, RH-53,866

REPORT NUMBER: 84R-078

SPONSOR: Rohm & Haas Co., Philadelphia, PA

TESTING FACILITY: Rohm & Haas Co., Toxicology Dept., Spring House, PA

TITLE OF REPORT: RH-3866: One Year Dietary Study in Beagle Dogs

AUTHOR(S): P.R. Goldman, J.C. Harris and J.D. Frantz

REPORT ISSUED: October 15, 1986

IDENTIFYING VOLUME: Volume 14 of 47

CONCLUSION: NOEL 100 ppm (3.09 mg/kg/day for males and 3.83 mg/kg/day for females) based upon hepatocellular hypertrophy. Supporting effects in organ weights and clinical chemistry observed. LOEL 400 ppm (14.28 mg/kg/day for males and 15.68 mg/kg/day for females)

Classification: CORE MINIMUM because full histopathology examinations were not submitted on the mid- and low dose levels.

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: alpha-butyl-alpha-4-chlorophenyl-1-H-1,2,4-triazole-propanenitrile
Description: white solid
Batch #(s), Other #(s): Lot No. 83159-7, Sample No. (TD No.) 84-063
Purity: 91.4%
Source: Rohm & Haas

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): male and female beagle dogs
Age: 5 months at start of test
Source(s): Marshall Research Animals (North Rose, NY)

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3. Procedure:

a. Dietary Preparation (if applicable): RH-3866 was heated until liquified, stirred, weighed, dissolved in acetone and blended with the feed. The acetone was evaporated off.

Frequency of preparation: weekly

Storage conditions: Stored at room temperature in jars (one jar per weekly diet preparation)

Stability Analyses: a sample from each weekly preparation was taken for analysis. Select samples were analyzed.

Homogeneity Analyses: The first time the diets were prepared, samples were taken from the top, middle, and bottom of each dietary concentration and submitted for analysis.

Concentration Analyses: This was done in connection with the stability and homogeneity analyses.

b. Basis For Selection of Dosage Levels:

Doses selected on the basis of a one-month range-finding study.

c. Animal Assignment and Dose Levels:

Test Group	Dose Administered ppm	Main Study 12 months male	female
Contr.	0	6	6
1	10	6	6
2	100	6	6
3	400	6	6
4	1600	6	6

d. Procedures for Studies Other Than Feeding and/or Additions, Changes in Feeding Study: Control and test diets were offered for same two hours per day.

e. Clinical Observations and Mortality: Dogs were observed daily for clinical signs of toxicity. Physical exams conducted weekly for first month and biweekly for remainder of treatment period.

f. Body Weight Determinations: Weekly

g. Food and/or Water Consumption: daily. Mean feed consumption per group calculated weekly.

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h. Ophthalmological Examinations (if applicable): Conducted during pre-test period and during weeks 26 and 52 of treatment period.

i. Clinical Pathology: (*) recommended by Guidelines

1) Hematology:

Collection times for blood (including # of animals):
weeks -2, -1, 13, 25, 39 and 53

The following CHECKED (X) parameters were examined:

X		X	
x	Hematocrit (HCT)*	x	Mean corpustular HGB (MCH)
x	Hemoglobin (HGB)*	x	Mean corpustular HGB conc.(MCHC)
x	Leukocyte count (WBC)*	x	Mean corpustular volume (MCV)
x	Erythrocyte count (RBC)*	x	Red blood cell morphology
x	Platelet count*		
	Total plasma protein (TP)		
x	Leukocyte differential count*		

2) Clinical Chemistry:

The following CHECKED (X) parameters were examined:

X	Electrolytes:
x	Calcium*
	Chloride*
	Magnesium*
x	Phosphorus*
	Potassium*
	Sodium*

X	Enzymes:
x	Alkaline phosphatase
	Cholinesterase
	Creatinine phosphokinase*
	Lactic acid dehydrogenase
x	Serum alanine aminotransferase (also SGPT)*
x	Serum aspartate aminotransferase (also SGOT)*
x	Gamma glutamyl transpeptidase

X	Other:
x	Albumin*
x	Blood creatinine*
x	Blood urea nitrogen*
x	Cholesterol*
x	Globulins
x	Glucose*
x	Total bilirubin*
x	Total protein*
	Triglycerides
x	A/G ratio

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3) Urinalysis:

Collection times for urine (including # of animals):
On each of 2 consecutive days at weeks -4 (all animals), 25 and 51 weeks (controls and high dose)

The following CHECKED (X) parameters were examined:

X		X	
x	Appearance*	x	Glucose*
	Volume*	x	Ketones*
x	Specific gravity*	x	Bilirubin*
x	pH	x	Blood*
x	Sediment (microscopic)*		Nitrate
x	Protein*		Urobilinogen

j. Gross Necropsy:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to end of exposure period and were subjected to complete gross pathological examinations:

None were sacrificed and none died prior to completion of the study.

Animals (groups) sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations:

All animals in all dose groups.

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k. Histopathology:

Animals (groups) which were sacrificed at the end of the treatment/observation period and were subjected to microscopic examination:

All animals from high dose and control groups; liver, gallbladder and testes in all dogs from all dose groups. Tissues were preserved from all animals from all dose groups for possible future examination.

CHECKED (X) tissues were preserved for histopathological examination and (XX) tissues were weighed upon removal from the animal. The (*) tissues were recommended by the Guidelines.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue	x	Aorta*	xx	Brain*
x	Salivary glands*	xx	Heart*	x	Periph. nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)*
x	Stomach*	x	Lymph nodes*	xx	Pituitary*
x	Duodenum*	xx	Spleen*	x	Eyes (optic n.)*
x	Jejunum*	x	Thymus*		Glandular
x	Ileum*		Urogenital	xx	Adrenals*
x	Cecum*	xx	Kidneys*		Lacrimal gland
x	Colon*	x	Urinary bladder*	x	Mammary gland*
x	Rectum*	x	Testes*	xx	Parathyroids*
xx	Liver*		Epididymides	xx	Thyroids*
x	Gall bladder*	x	Prostate		Other
x	Pancreas*	x	Seminal vesicle	x	Bone*
	Respiratory	x	Ovaries	x	Skeletal muscle*
x	Trachea*	x	Uterus*	x	Skin
x	Lung*			x	All gross lesions and masses

1. Statistical Analyses: Distributions of all continuous data were inspected for normality and homogeneity of variance across treatment groups. Analyses of variance were used when needed. Duncan's multiple range test was used on some data as well as T-tests.

B. RESULTS:

1. Dietary Preparation: Average dose levels ranged between 97-111% of the theoretical dose levels, with an overall average of 103%.

2. Clinical Observations and Mortality: No deaths were observed and no clinical signs of toxicity were noted at any of the dose levels throughout the study.

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3. Body Weight Determinations: The body weights of male dogs at the highest dose level were significantly decreased following one week of treatment but were similar to control values throughout the remainder of the study. The mean body weights of female dogs at this dose level were significantly less than the control values for the first 5 weeks of the study. No treatment related changes were noted in the body weights of either sex at any of the other dose levels.

4. Food and/or Water Consumption: Food consumption of female dogs fed the highest dose level was consistently below that of the controls throughout the study. Food consumption of males at the highest dose level was below that of the controls during the first week but was similar to controls during the remainder of the study. No differences were observed in the food consumption of the other groups when compared to controls.

5. Ophthalmological Examinations: No ophthalmological abnormalities were seen in any of the treated dogs.

6. Hematology: A slightly decreased number of red blood cells (RBC), an increased number of platelets, an increase in mean cell hemoglobin and an increase in the mean corpuscular volume were observed in male dogs at the highest dose level throughout the treatment period. A slight increase in the mean cell hemoglobin was observed in male dogs at 400 ppm. No other treatment related changes (other than spurious differences) were observed in any of the other parameters or in any of the other dose groups.

7. Clinical Chemistry: Increases in inorganic phosphorus and alkaline phosphatase and a decrease in serum albumin were observed in both sexes at the highest dose level. Alkaline phosphatase was also increased in females at 400 ppm. SGPT was increased in males and GGT was increased in females at the highest dose level (1600 ppm). These changes were consistent throughout the treatment period. No other consistent changes were noted in any of the other parameters or in any of the other dose groups.

8. Urinalysis: No treatment related changes were noted in any of the treated groups.

9. Gross Pathology: Gross changes were observed in the livers of high dose dogs of both sexes. These changes consisted of enlargement and/or accentuated lobular architecture (1 male and 3 females). Other changes were considered incidental and were found in all groups, including controls. Frequent changes included reddened portions of the intestinal tract, thickened and reddened mammary glands (females only) and distended uteri (females only).

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10. Organ Weights: Increased absolute and relative liver weights were observed in both sexes at 1600 ppm and in females at 400 ppm. No other observed changes in organ weights were considered to be related to treatment. The statistically significant increase in absolute liver weight of 100 ppm female dogs is considered to be due to the larger size of the dogs in this group when compared to control dogs and the statistically significant increase in relative liver weights in the 10 ppm dogs is considered to be due in part to the smaller size of the dogs at this dose level.

11. Histopathology:

    a. Nonneoplastic lesions: Compound-related nonneoplastic lesions were observed in the livers of both sexes of dogs from the 400 ppm and from the 1600 ppm dose groups. These lesions included minimal to mild hepatocellular hypertrophy in 1/6 of the 400 ppm male dogs and in 5/6 of the 1600 ppm male dogs and mild to moderate hepatocellular hypertrophy in 2/6 of the 400 ppm female dogs and in 6/6 of the 1600 ppm female dogs. The hypertrophy was characterized by cells with large amounts of pale, eosinophilic, finely granular cytoplasm; in a few more severely affected livers it was noted to be almost panlobular in distribution. "Ballooned" hepatocytes or expanded hepatocytes with large clear cytoplasmic spaces, sometimes containing only a few strands of pink cytoplasm were sporatically observed in some of the more severely affected high dose females. These cells were thought to represent severely hypertrophied, possibly degenerating, hepatocytes. No changes in the liver related to treatment were noted in any of the animals in the lower dose levels. Table 1 summarizes the changes noted in the livers of the treated animals. No treatment related changes were observed in the testes of any of the male animals. Incidences of lesions in treated animals were similar to controls at all dose levels. Changes in other organs were considered to be incidental and not related to treatment.

    b. Neoplastic lesions: No neoplastic lesions were observed in any of the animals.

12. Quality Assurance Measures: The report and the original data from the study were reviewed for adherence to the GLP's and the study was audited a number of times throughout the pretreatment and treatment phases. The report is signed by the Quality Assurance Unit.

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C. DISCUSSION: This study is in general well conducted. There is, however, one important item missing: a full microscopic examination was not conducted on the mid- and low dose animals. Full microscopic examinations on all animals in nonrodent studies are required by the EPA Guidelines. In addition to incomplete microscopic examinations, there were a few minor missing items in the hematology summary (no mention of the statistically significant increases in mean cell hemoglobin in the top two dose levels and an increase in mean corpuscular volume at the top dose level). The study is CORE MINIMUM because complete microscopic examinations were not submitted on the mid- and low dose levels. In this case, the study was excepted because no other effects were seen in any of the other chronic studies except testicular effects and the testes were examined microscopically at all dose levels in the study. It is not likely that complete microscopic examinations at all dose levels would change the outcome of the study. The NOEL is 100 ppm (3.09 mg/kg/day for males and 3.83 mg/kg/day for females) based upon hepatocellular hypertrophy and the LOEL is 400 ppm (14.28 mg/kg/day for males and 15.68 mg/kg/day for females). Supporting effects were observed as well.

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RIN 1067-98

Myclobutanil Tox Review

Page 42 is not included in this copy.

Pages _____ through _____ are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

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____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

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Reviewed By: Pamela Hurley, Toxicologist Pamela M. Hurley 4/5/94
Section I, Tox. Branch (7509C)
Secondary Reviewer: Roger L. Gardner, Section Head
Section I, Tox. Branch (7509C) Roger Gardner 4/7/94

011124

DATA EVALUATION RECORD

STUDY TYPE: Oncogenicity Study in Mice (83-2)

SHAUGHNESSY NO./TOX. CHEM. NO.: 128857 / 723K

ACCESSION NO./MRID NO.: 428091-02

DP BARCODE NO.: D193334, D193309, D193312, D193322, D193332

TEST MATERIAL: Myclobutanil

SYNONYMS: Rally®, Nova®, Eagle®

LABORATORY PROJECT I.D.NUMBER(S): 89R-261

SPONSOR: Rohm and Haas Company, Spring House, PA 19477

TESTING FACILITY: Rohm and Haas Toxicology Department, 727 Norristown Road, Spring House, PA 19477

TITLE OF REPORT: RH-3866: Dietary Oncogenicity Study in Female Mice

AUTHOR(S): D. M. Anderson, G. P. O'Hara, W. R. Brown

REPORT ISSUED: 3/17/93

CONCLUSION: In an 18-month carcinogenicity study, technical myclobutanil (92.9%) was administered to 60 female Crl:CD®-1(ICR)BR mice at dose levels of 0 or 2000 ppm (393.5 mg/kg/day) in the diet. Ten from each group were sacrificed at 52 weeks. An additional 20 animals were added to the study as sentinels and also sacrificed at 52 weeks. This study was conducted at the request of the HED Carcinogenicity Peer Review Committee because it was determined that the previous study (MRID No. 00164990) was not conducted at sufficiently high dose levels in females. It was agreed that the mouse carcinogenicity study should be repeated in females with the highest dose approaching the "maximum tolerated dose" level (e.g., about 2000 ppm).

At 2000 ppm, statistically significant decreases in body weight (2 - 12%) and body weight gain (12 - 26%); increases in liver weights (22.9 - 33.2%); hepatocellular hypertrophy; hepatocellular vacuolation; necrosis of single hypertrophied hepatocytes; yellow-brown pigment in the Kupffer cells and cytoplasmic eosinophilia and hypertrophy of the cells of the zona fasciculata area of the adrenal cortex. With the exception of the pigment in the Kuppfer cells, these lesions were present at

the 12-month sacrifice. **In this study, the NOEL could not be established because there were effects at the only dose level tested (decreases in body weight and body weight gain; increases in liver weights; hepatocellular hypertrophy; hepatocellular vacuolation; necrosis of single hypertrophied hepatocytes; yellow-brown pigment in the Kupffer cells and cytoplasmic eosinophilia and hypertrophy of the cells of the zona fasciculata area of the adrenal cortex).**

Myclobutanil was not oncogenic when tested on Crl:CD®-1(ICR)BR mice under the conditions of the study. However, it is noted that this study is an 18-month study whereas the old study is a 24-month study.

The study is classified as Core Minimum when used in conjunction with the previously conducted study (MRID No. 00164990, executive summary below, DER attached). The two studies together satisfy the regulatory requirement for an oncogenicity study in the mouse (83-2).

In the previously conducted study, technical myclobutanil (90.4%) was administered to 110 male and 110 female Crl:CD®-1(ICR)BR mice in the diet for a period of 24 months at the following dose levels: 0, 20, 100 or 500 ppm. The overall calculated mean daily compound consumption was 0, 2.7, 13.7 or 70.2 mg/kg/day for males and 0, 3.2, 16.5 or 85.2 mg/kg/day for females. Of the 110 animals/dose group, ten/sex/dose group were sacrificed at 3 and 6 months and 20/sex/dose group were sacrificed at 12 months. A sentinel animal program was also conducted separately in which 25 animals/sex were evaluated at 24 months.

At 100 ppm, an increase in hepatic mixed-function oxidase (MFO) was observed at 3 months in females (by 29% per mg protein, by 34% per g liver; $p \leq 0.05$). At 500 ppm the following was observed: an increase in MFO in both sexes at 3 (119% (♂) and 61% (♀) above control per total liver) and 6 months (212% (♂) and 210% (♀) above control per total liver) and in females at 12 months (266% above control per total liver); an increase in SGPT values in females at 3 months (59% above controls) and an increase in absolute and relative liver weights in males (10%) and in females (12% and 13%, respectively) at 3 months. In addition, the following treatment-related changes were observed microscopically after 3, 6 and 12 months of treatment in males: increased incidence and severity of centrilobular hepatocytic hypertrophy, Kupffer cell pigmentation, periportal punctate vacuolation and individual hepatocellular necrosis. Following 24 months of treatment, increased incidences of focal hepatocellular alterations and multifocal hepatocellular vacuolation were observed in both sexes. These were not associated with any hypertrophy, hyperplasia or neoplastic proliferations in the liver. **At the time the DER was written, the LEL was considered to be 100 ppm (16.5 mg/kg/day) based on an increase in hepatic**

mixed-function oxidase (MFO) at 3 months in females. The NOEL was 20 ppm (3.2 mg/kg/day). The increase in MFO at 3 months in females is not considered to be a significant enough effect to establish an LEL. Therefore, the LEL is raised to 500 ppm (70.2 mg/kg/day for males and 85.2 mg/kg/day for females) based on increases in MFO in both sexes; increases in SGPT values in females and in absolute and relative liver weights in both sexes at 3 months; increased incidences and severity of centrilobular hepatocytic hypertrophy, Kupffer cell pigmentation, periportal punctate vacuolation and individual hepatocellular necrosis in males; and increased incidences of focal hepatocellular alterations and multifocal hepatocellular vacuolation in both sexes. The NOEL is 100 ppm (16.5 mg/kg/day).

The study is classified as Core Minimum when used in conjunction with the present study. The two studies together satisfies the regulatory requirement for an oncogenicity study in the mouse (83-2).

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: 2-Butyl-2-(4-chlorophenyl)-1H-1,2,4-triazole-1-propane-nitrile

Description: Almond colored solid
Batch #(s), Other #(s): Lot # 2-2943
Purity: 92.9%
Source: Rohm & Haas
Vehicle: Acetone

2. Test Animals:

Species and Strain (sexes): Male and female Crl:CD®-1(ICR)BR female mice
Age: 3 weeks at receipt (acclimated 3 weeks prior to administration of test diets).
Weight(s): 23.9 g at start of study.
Source(s): Charles River Laboratories, Inc., Raleigh, North Carolina
Housing: Individually in stainless-steel, hanging wire-mesh cages.

3. Procedure:

a. Dietary Preparation: The test material was heated to approximately 70°C until liquified and stirred to ensure apparent homogeneity. The required amount of test material was then weighed, dissolved in actone and mixed with approximately 1 kg of untreated feed and blended in a hood to

untreated feed and blended an additional 15 minutes. The control diet was prepared in the same manner.

Frequency of preparation: Every 2 weeks until it was determined that the test material was stable in the diet for 28 days at room temperature. It was then prepared every 4 weeks.

Storage conditions: Stored at room temperature.

Stability and Homogeneity Analyses: When the diet was first prepared, samples were taken from the top, middle and bottom of the feed at both dietary concentrations and submitted for analysis of active ingredient. Twenty-eight day retention samples were also analyzed to determine stability of the diet at room temperature.

Concentration Analyses: Samples of the diets prepared in weeks 5, 9, 13, 17, 21, 25, 29, 37, 49, 61, 73 and 77 were taken and analyzed for content of active ingredient.

b. Basis For Selection of Dose Levels: Dietary levels were selected on the basis of the results from a previously submitted chronic feeding/oncogenicity study in mice. From that study, the Agency had determined that 2000 ppm was considered to be an appropriate high dose for females. Therefore, for this study, the Registrant was only required to test one dose level in females. This study would then be evaluated along with the previous mouse study.

c. Animal Assignment and Dose Levels:

Test Group	Dose Administered ppm	Main Study 18 months male	Main Study 18 months female	Interim Sac. 52 weeks male	Interim Sac. 52 weeks female
1 (control)	0	-	50	-	10
2	2500	-	50	-	10
3 (sentinel)	0	-	-	-	20

d. Additions and/or Alterations in Procedure:

The 20 females that were selected for the sentinel program received basal diet only. Otherwise, they were handled in the same manner as the other test animals.

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e. Clinical Observations and Mortality: The animals were observed for clinical signs of toxicity and moribundity daily. A thorough physical examination, including palpation for tissue masses and evaluation of external structures, posture, gait and behavior were conducted weekly. Abnormalities in urine and feces, respiration and body temperature were also noted.

f. Body Weight Determinations: Body weights were measured prior to the start of the study, weekly through week 13 and monthly thereafter.

g. Food and/or Water Consumption: Food consumption was measured and recorded weekly through week 13 and monthly thereafter. Compound consumption values were also calculated.

h. Ophthalmological Examinations: Not conducted.

i. Clinical Pathology: (*) recommended by Guidelines

1) Hematology:

Collection times for blood (including # of animals): Blood samples were collected from all mice euthanized after 12 or 18 months on test for white blood cell count and differential evaluation. Prior to blood collection, all mice were fasted for at least 1 hour. The mice were anesthethized with an i.p. injection of pentobarbital prior to blood collection. Blood was collected via tail clipping. The parameters monitored were total and differential white blood cell counts.

2) Clinical Chemistry:

Clinical chemistry studies were not conducted.

3) Urinalysis: Urinalysis studies were not conducted.

j. Gross Necropsy:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to end of exposure period and were subjected to complete gross pathological examinations: All animals.

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Animals (groups) sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations: All animals.

The necropsies included examination of all orifices, organs and tissues.

k. Histopathology:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to the end of the exposure period and were subjected to microscopic examination: all animals.

Animals (groups) which were sacrificed at the end of the treatment/observation period and were subjected to microscopic examination: All animals.

CHECKED (X) tissues were preserved for histopathological examination and (XX) tissues were weighed upon removal from the animal. The (*) tissues were recommended by the Guidelines.

X	
	Digestive system
x	Tongue
x	Salivary glands*
x	Esophagus*
x	Stomach*
x	Duodenum*
x	Jejunum*
x	Ileum*
x	Cecum*
x	Colon*
x	Rectum*
xx	Liver*
x	Gall bladder*
x	Pancreas*
	Respiratory
x	Trachea*
x	Lung*
x	Larynx
x	Hardarian gland

X	
	Cardiovasc./Hemat.
x	Aorta*
x	Heart*
x	Bone marrow* (femur & sternum)
x	Lymph nodes*
x	Spleen*
x	Thymus*
	Urogenital
xx	Kidneys*
x	Urinary bladder
	Testes*
	Epididymides
	Prostate
	Seminal vesicle
x	Ovaries
x	Uterus* with vagina

X	
	Neurologic
xx	Brain*
x	Periph. nerve*
x	Spinal cord (3 levels)*
x	Pituitary*
x	Eyes (optic n.)*
	Glandular
xx	Adrenals*
	Lacrimal gland
x	Mammary gland*
x	Parathyroids*
x	Thyroids*
	Other
x	Bone* (femur, sternum)
x	Skeletal muscle*
x	Skin
x	All gross lesions and masses

1. Statistical Analyses: Survival distributions were compared using SAS PROC LIFETEST. The Wilcoxin test and the log rank test were used to assess differences in the distribution of survival times. Mean absolute body weights, body weight changes, total food consumption, and organ weight data were statistically analyzed by inspecting for normality and homogeneity of variance. A square root transformation was applied to the white blood cell differential count data prior to further analysis. One-way analysis of variance models (or covariance models when pre-test values were available) were used to assess the presence of absence of a treatment effect at each sampling time. Group means were compared using a least squared means. Comparisons of the incidence of neoplastic and non-neoplastic findings were performed using a two-sided Fisher's exact test whenever a difference of two or more findings was noted between the treated group and the control group. Fisher's exact test for contingency tables was used to compare the severity of treatment-related histopathologic findings between the two groups.

B. RESULTS:

1. Dietary Preparation: Results from the stability analyses indicate that the test formulation was stable up to 28 days. The homogeneity studies indicated homogeneous mixing. The average homogeneity % of targets ranged from 103 to 113%. The average stability results ranged from 100 - 105% and the concentration analyses indicated that the formulations averaged from 84.7 - 104% of the target dose. The coefficient of variation was within 10%.

2. Clinical Observations and Mortality: There were no indications of infectious diseases in the sentinel animals. There was no apparent compound-related effect on survival. The following table summarizes the survival for the control and treated groups.

Survival Data for Female Mice

Week	0 ppm	2000 ppm
0	59/60	60/60
17	59/60	59/60
30	58/60	56/60
40	58/60	54/60
52	46/50	43/50
66	38/50	38/50
73	33/50	35/50
76	32/50	33/50

[a]( ) = % survival from original number in group.

No treatment-related clinical signs of toxicity were observed in the treated groups when compared to the controls. A summary table was not provided in the report. However, the individual animal data indicate that the following clinical signs were some of those observed in animals in both groups: alopecia, scabs, facial swelling, yellow staining of fur, ear and paw lacerations, occasional eye opacities and tremors (only 2 - 3 animals).

3. Body Weight Determinations: Mean body weight and cumulative body weight were significantly decreased in the treated group. The mean body weight was deceased 2 - 12% of the control and the cumulative body weight change was decreased 12 - 26% of the control over the course of the study. The following table summarizes body weight gain.

Mean Body Cumulative Weight Change (g)

Dose (ppm)	Week	0	4	8	12	25	37	57	77
0	Mean	1.3	5.4	7.4	8.9	10.9	13.1	16.1	15.8
2000	Mean	1.4	4.4*	5.7*	7.2*	8.9*	11.0*	12.0*	12.8*
	% Control	108	81	77	81	82	84	75	81

* Significantly different from control value, $p \leq 0.05$.

4. Food and/or Water Consumption: Food consumption was generally decreased throughout the study. Mean food consumption was statistically significantly decreased during weeks 1, 2, 5, 21, 25, 41 and 49. Only one of

these values was less than 90% of the control values. The following table summarizes the results for these weeks.

Mean Food Consumption (g)

Dose (ppm)	Week	1	2	5	21	25	41	49
0	Mean	45.0	47.3	49.3	44.7	47.0	42.8	42.3
2000	Mean	42.6*	43.4*	45.1*	40.8*	41.5*	38.5*	36.2*
	% Control	95	92	91	91	88	90	86

5. Hematology: An increase in mean white blood cell count was observed at 12 (6.1 versus 9.3 $10^3/mm^3$ and 18 months (5.9 versus 8.0 $10^3/mm^3$, $p < 0.05$). It was only statistically significant at 18 months. The authors of the report believed that the increase was not toxicologically significant because there was no histologic evidence of treatment-related inflammatory processes or blood disorders.

6. Gross Pathology: No treatment-related findings were observed.

7. Organ Weights: Statistically significant increases in absolute and relative liver weights were observed at 12 and 18 months. At 18 months, statistically significant increases in relative brain weights and absolute kidney weights were also observed. The following table summarizes the results.

Selected Organ Weights at Interim and Terminal Sacrifices

	Interim		Terminal	
Organ	0 ppm	2000 ppm	0 ppm	2000 ppm
Liver				
Absolute	1.900	2.467	2.026	2.491*
Relative	500.72	662.68*	525.07	699.59*
Brain				
Absolute	0.547	0.524	0.539	0.543
Relative	145.65	141.53	141.72	153.74*
Kidney				
Absolute	0.545	0.497	0.550	0.498*
Relative	143.91	133.27	143.17	140.18
Adrenal				
Absolute	0.009	0.009	0.009	0.008
Relative	2.31	2.54	2.42	2.40

* Significantly different from control value, $p \leq 0.05$.
Absolute = grams; relative = organ weight x 10,000/ body weight

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8. Histopathology:

a. Nonneoplastic lesions: In animals which were sacrificed at 12 months, treatment-related changes were observed in the liver and adrenal glands. In the liver, hepatocellular hypertrophy was noted and in the adrenal, increased cytoplasmic eosinophilia and hypertrophy of the cells of the zona fasciculata area of the cortex were observed. The report stated that the hepatocellular hypertrophy "was diffuse with hepatocytes in all areas of the lobules being enlarged, but the most prominent enlargement frequently was in the centrilobular areas. Some of the enlarged hepatocytes had an increased amount of a pale, granular and/or vacuolated cytoplasm (hepatocellular vacuolation). Necrosis of single hypertrophied hepatocytes was observed in one of the affected compound-treated mice."

In animals which did not survive to the terminal sacrifice and in animals which were sacrificed at 18 months, the "incidence and/or severity of the hepatocellular hypertrophy and vacuolation generally were increased...In addition to the hepatocellular hypertrophy, a few compound-treated mice (3/17 nonsurviving mice and 2/33 surviving mice) had an increased amount of yellow-brown pigment in Kupffer cells and macrophages scattered throughout the liver. Necrosis of single enlarged hepatocytes was seen in five compound-treated mice (two nonsurviving and three surviving mice). Single-cell necrosis not associated with hepatocellular hypertrophy occurred in one control mouse sacrificed at termination of the study."

An increased incidence of hypertrophy of the cells of the zona fasciculata of the adrenal cortex was also observed in treated mice. This effect was also seen in 6 control mice which did not survive until termination of the study. The following table summarizes the incidences of selected non-neoplastic lesions in mice at 12 months, in mice that died prior to termination, in mice sacrificed at 18 months and in all mice. The table after that summarizes the severity of the liver and adrenal lesions in all mice.

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Incidences of Selected Non-neoplastic Lesions at Various Sacrifice Times

Lesion	0 ppm	2000 ppm
12 Months		
Adrenal gland		
# Examined	10	10
Hypertrophy, cortex	0	2
Liver		
# Examined	10	10
Hepatocellular hypertrophy	0	10
Hepatocellular vacuolation	0	5
Nonsurviving Mice		
Adrenal gland		
# Examined	18	17
Hypertrophy, cortex	6	7
Kidneys		
# Examined	18	17
Infiltration, mononuclear-cell, focal	4	7
Liver		
# Examined	18	17
Hepatocellular hypertrophy	2	15
Necrosis, single cell	0	2
Pigment, yellow-brown	0	3
Hepatocellular vacuolation	0	2
Thymus		
# Examined	18	17
Atrophy	0	3
18-Month Terminal Sacrifice		
Adrenal		
# Examined	32	33
Hypertrophy of the cortex	0	8
Liver		
# Examined	32	33
Hepatocellular hypertrophy	1	33
Necrosis, single cell	1	3
Pigment, yellow-brown	0	2
Hepatocellular vacuolation	0	22
Ovaries		
# Examined	32	33
Cystic bursa(e)	6	9
Hematocyst(s)	0	2

Incidences of Selected Non-neoplastic Lesions at Various Sacrifice Times

Lesion	0 ppm	2000 ppm
Tongue		
# Examined	32	33
Periarteritis	1	4
Urinary bladder		
# Examined	32	33
Infiltration, mononuclear-cell, focal	4	9
All Animals		
Adrenal glands		
# Examined	60	60
Hypertrophy, cortex	6	17
Liver		
# Examined	59	60
Hepatocellular hypertrophy	3	58
Pigment, yellow-brown	0	5
Hepatocellular vacuolation	0	29

Incidence and Degree of Severity of Histomorphologic Observations - All Mice

Observation	0 ppm	2000 ppm
Adrenal gland		
# Examined	60	60
Hypertrophy, cortex	6	17
minimal	0	4
slight	5	5
moderate	0	8
marked	1	0
Liver		
# Examined	60	60
Hepatocellular hypertrophy	3	58
minimal	1	8
slight	2	24
moderate	0	26
Single-cell necrosis	1	6
minimal	0	4
slight	0	2
moderate	1	0
Vacuolation, hepatocellular	0	29
minimal	0	3
slight	0	15
moderate	0	11

b. Neoplastic lesions: No treatment-related neoplastic lesions were observed. The following table summarizes selected neoplastic lesions of interest.

Selected Neoplastic Lesions - All Animals

Lesion	0 ppm	2000 ppm
Liver		
# Examined	60	60
Hepatocellular adenoma	0	1
Hepatocellular carcinoma	1	0
Mammary Gland		
# Examined	57	59
Adenocarcinoma	1	0
Ovaries		
# Examined	60	60
Leiomyosarcoma	0	1
Pituitary		
# Examined	59	60
Adenocarcinoma, pars distalis	1	0
Adenoma, pars distalis	1	0
Uterus		
# Examined	60	60
Adenoma, endometrial	1	0
Hemangiosarcoma	1	0
Leiomyosarcoma	1	0

9. Quality Assurance Measures: Signed Good Laboratory Practice Statement and Quality Assurance Statements were provided.

C. DISCUSSION: This study was conducted at the request of the Toxicology Branch (TB-I) because it was determined that the previous study (MRID No. 00164990) was not conducted at sufficiently high dose levels. On February 9, 1988, the then Toxicology Branch Peer Review Committee met to review the toxicology data base on myclobutanil (see memorandum from J. Quest to L. Rossi, dated 3/1/88). At that meeting, it was agreed that the mouse chronic feeding/oncogenicity study should be repeated in females with the highest dose approaching the "maximum tolerated dose" level (e.g., about 2000 ppm). This study fulfills that request.

The NOEL cannot be calculated from this study because there were effects at the one dose level tested (2000 ppm or 393.5 mg/kg/day). Therefore, the NOEL from the previously conducted study will be used: 100 ppm (16.5 mg/kg/day).

The LEL is 500 ppm (70.2 mg/kg/day for males and 85.2 mg/kg/day for females) based on increases in MFO in both sexes; increases in SGPT values in females and in absolute and relative liver weights in both sexes at 3 months; increased incidences and severity of centrilobular hepatocytic hypertrophy, Kupffer cell pigmentation, periportal punctate vacuolation and individual hepatocellular necrosis in males; and increased incidences of focal hepatocellular alterations and multifocal hepatocellular vacuolation in both sexes.

The effects observed in this study were similar to those observed in the previous study with a few additions: decrease in body weight and body weight gain; increases in liver weights; hepatocellular hypertrophy; hepatocellular vacuolation; necrosis of single hypertrophied hepatocytes; yellow-brown pigment in the Kupffer cells and cytoplasmic eosinophilia and hypertrophy of the cells of the zona fasciculata area of the adrenal cortex.

This study, when taken in conjunction with the previously conducted study satisfies the regulatory requirement for an oncogenicity study in the mouse. Myclobutanil was not oncogenic under the conditions of the study.

Reviewed by: Pamela Hurley
Section 2 , Tox. Branch (TS-769C)
Secondary Reviewer: Edwin Budd
Section 2 , Tox. Branch (TS-769C)

11/11/88

DATA EVALUATION REPORT

STUDY TYPE: Chronic/Oncogenicity - Mouse (83-5) TOX. CHEM. NO.: 723K

ACCESSION NUMBER: 266090

TEST MATERIAL: RH-3866

SYNONYMS: Rally, Systhane, Myclobutanil

REPORT NUMBER: 84R-023

SPONSOR: Rohm & Haas Company, Philadelphia, PA

TESTING FACILITY: Toxicology Dept., Rohm & Haas Company, Spring House, PA

TITLE OF REPORT: RH-3866: Dietary Chronic and Oncogenicity Study in Mice

AUTHOR(S): P.R. Goldman and J.C. Harris

REPORT ISSUED: October 17, 1986

IDENTIFYING VOLUME: Volume 16 of 47

CONCLUSION: The NOEL was 20 ppm and the LOEL was 100 ppm (slight increase in liver mixed function oxidase). Microscopic changes in the liver were evident in both sexes at 500 ppm.

Classification: CORE GUIDELINE for chronic effects and CORE SUPPLEMENTARY for oncogenicity (see discussion).

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: alpha-butyl-alpha-4-chlorophenyl-1H-1,2,4-triazole-1-propanenitrile
Description: red-brown solid
Batch #(s), Other #(s): Sample # 83-260, lot # LAP-0298
Purity: 90.4%
Source: Rohm & Haas

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): Male and female Crl:CD®-1(ICR)BR mice
Age: 3 weeks upon receipt
Source(s): Charles River Breeding Labs

3. Procedure:

a. Dietary Preparation (if applicable): Sample was heated to approximately 70°C until liquified. Liquid was stirred to ensure homogeneity, weighed, dissolved in acetone and mixed with feed in a hood to evaporate the acetone.
Frequency of preparation: weekly
Storage conditions: at room temperature, in dark dry area
Stability Analyses: Each week, an extra feed cup for each dietary level was prepared and left on top of the cage rack in the study room for the treatment week, collected and then submitted for analysis in order to verify stability.
Homogeneity Analyses: The first time the diet was prepared, samples from the top, middle and bottom were taken for analysis.
Concentration Analyses: all samples obtained to assess adequacy of mixing and those obtained during first month for quality assurance were analyzed as well as one sample from each dietary concentration per month. Other samples were preserved and sent to analysis group.

b. Basis For Selection of Dosage Levels:

Not stated, but probably based upon results of subchronic study that was conducted.

c. Animal Assignment and Dose Levels:

Test Group	Dose Administered ppm	Main Study 24 months		Interim Sac. 3 months		Interim Sac. 6 months		Interim Sac. 12 months	
		male	female	male	female	male	female	male	female
Contr.	0	70	70	10	10	10	10	20	20
1	20	70	70	10	10	10	10	20	20
2	100	70	70	10	10	10	10	20	20
3	500	70	70	10	10	10	10	20	20
4	Sentinel	25	25	-	-	-	-	-	-

d. Clinical Observations and Mortality: Animals observed daily for signs of ill health and reaction to treatment. Physical exams conducted weekly for first 14 weeks and at 2 week intervals thereafter.

e. Body Weight Determinations: weekly

f. Food and/or Water Consumption: weekly

g. Ophthalmological Examinations (if applicable): 12 and 24 months

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h. Clinical Pathology: (*) recommended by Guidelines

1) Hematology:

Collection times for blood (including # of animals):
3, 6, 12 and 24 months; 10/sex/group at 3 and 6 months, 15/sex/group at 12 and 24 months.

The following CHECKED (X) parameters were examined:

X		X	
x	Hematocrit (HCT)*	x	Mean corpuscular HGB (MCH)
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB conc.(MCHC)
x	Leukocyte count (WBC)*	x	Mean corpuscular volume (MCV)
x	Erythrocyte count (RBC)*	x	Red cell morphology†
x	Platelet count*		
	Total plasma protein (TP)		
x	Leukocyte differential count*†		†Only on high dose and controls

2) Clinical Chemistry:

The following CHECKED (X) parameters were examined:

X	Electrolytes:	X	Other:
x	Calcium*	x	Albumin*
	Chloride*	x	Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
x	Phosphorus*	x	Cholesterol*
	Potassium*	x	Globulins
	Sodium*	x	Glucose*
	Enzymes:	x	Total bilirubin*
x	Alkaline phosphatase	x	Total protein*
	Cholinesterase	x	Triglycerides
	Creatinine phosphokinase*	x	A/G ratio
	Lactic acid dehydrogenase		
x	Serum alanine aminotransferase (also SGPT)*		
x	Serum aspartate aminotransferase (also SGOT)*		
x	Gamma glutamyl transpeptidase		

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3) Urinalysis:

Collection times for urine (including # of animals): 6, 12 and 24 months from same animals as blood.

The following CHECKED (X) parameters were examined:

X		X	
x	Appearance*	x	Glucose*
	Volume*	x	Ketones*
x	Specific gravity*	x	Bilirubin*
x	pH	x	Blood*
x	Sediment (microscopic)*		Nitrate
x	Protein*		Urobilinogen

4) Hepatic Mixed Function Oxidase (MFO) and Peroxisomal Beta-Oxidation Analyses

At 3, 6, and 12 months, livers from 6 mice/sex/group were randomly selected from animals scheduled for post-mortem examinations and analyzed for MFO activity. Additional samples taken from the 12 month sacrifice were frozen and subsequently analyzed for hepatic peroxisomal beta-oxidation activity.

i. Gross Necropsy:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to end of exposure period and were subjected to complete gross pathological examinations:

All animals.

Animals (groups) sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations:

All animals.

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j. Histopathology:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to the end of the exposure period and were subjected to microscopic examination:

Tissues were preserved from all animals. Only liver was examined microscopically from animals scheduled for sacrifice at 3 and 6 months. At 12 months, all tissues listed below examined for controls and high dose; liver, gross lesions and tissue masses were examined for other dose groups. All tissues examined for non-surviving mice in all dose groups.

Animals (groups) which were sacrificed at the end of the treatment/observation period and were subjected to microscopic examination:

At 24 months, all tissues examined in controls and high dose groups; liver, kidneys, lungs, testes and tissues with gross changes were examined in other dose groups. Tissues examined from sentinel mice were brain, liver, kidneys, lung, spleen, liver, colon and other tissues with gross changes.

CHECKED (X) tissues were preserved for histopathological examination and (XX) tissues were weighed upon removal from the animal. The (*) tissues were recommended by the Guidelines.

X	Digestive system
	Tongue
x	Salivary glands*
x	Esophagus*
x	Stomach*
x	Duodenum*
x	Jejunum*
x	Ileum*
x	Cecum*
x	Colon*
x	Rectum*
xx	Liver*
x	Gall bladder*
x	Pancreas*
	Respiratory
x	Trachea*
x	Lung*
x	Larynx

X	Cardiovasc./Hemat.
	Aorta*
xx	Heart*
x	Bone marrow*
x	Lymph nodes*
xx	Spleen*
x	Thymus*
	Urogenital
xx	Kidneys*
x	Urinary bladder*
xx	Testes*
x	Epididymides
x	Prostate
x	Seminal vesicle
xx	Ovaries
x	Uterus*
x	Vaginat
x	Coagulating glandt

X	Neurologic
xx	Brain*
x	Periph. nerve*
x	Spinal cord (3 levels)*
x	Pituitary*
x	Eyes (optic n.)*
	Glandular
xx	Adrenals*
	Lacrimal gland
x	Mammary gland*
x	Parathyroids*
x	Thyroids*
	Other
x	Bone*
x	Skeletal muscle*
x	Skin
x	All gross lesions and masses

† Saved at 12 and 24 months only. The coagulating gland/seminal vesicles/prostate glands were collected at necropsy as a single unit.

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k. Statistical Analyses: Body weights, feed consumption, clinical chemistry, hematology, urinalysis and organ weights inspected for normality and homogeneity of variance across treatment groups by examining residual plots. Analysis of variance used in assessments for overall treatment effect; group means compared using Duncan's test when significant treatment effect found. Survival distributions compared separately within each sex and also pooled over sex by using both logrank and Wilcoxon tests found in PROC LIFETEST of the Statistical Analysis System (SAS).

B. RESULTS:

1. Dietary Preparation: Samples for week 1 homogeneity analyses as well as samples retained from weeks 1, 2, 4 and those taken from 4 week intervals were analyzed for RH-3866 concentration. The overall average concentrations for each dose level ranged from 92-105% of the theoretical dosages. The average for the 3 concentrations together was 98%. The individual concentrations for each dose level ranged from 11-40 ppm for the 20 ppm dose level, from 57-130 ppm for the 100 ppm dose level and from 280-890 ppm for the 500 ppm dose level. Obviously, the extreme deviations from the theoretical dose levels did not occur very often.

2. Clinical Observations and Mortality: There was no apparent effect of the test chemical on the survival of the test groups. Percent survival at the end of the 24 month treatment period was 50, 47, 44, and 56% for the controls, 20, 100, and 500 ppm groups, respectively for males and 46, 51, 47 and 51% for the females, respectively. No treatment-related signs of clinical toxicity were observed in any of the test groups. The following signs were observed in all groups: red swollen ears, alopecia, arched back and yellow stained anogenital area. According to the authors, some mice showed some common pre-death signs associated with a debilitated state. These signs included ataxia, tremors and lethargy.

3. Body Weight Determinations: No treatment related changes in body weight were observed in any of the test groups. Significantly decreased body weights were observed when compared to controls at individual times in the highest dose group (only once in the mid-dose group in females after the pretest period), but these were not consistent.

4. Food and/or Water Consumption: No dose-related changes in food consumption were observed with any of the treated groups. Sporatic statistically significant increases and decreases in food consumption were observed in all of the treated groups.

5. Ophthalmological Examinations: No treatment-related abnormalities were observed in any of the treated groups.

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6. Hematology: No treatment-related changes were observed in any test group. A significant increase in the mean corpuscular hemoglobin concentration value was observed in female rats at the 20 ppm dose level at 6 months. This did not occur in any of the higher dose levels, nor did it occur again at any of the other time periods. Therefore, it was considered to be spurious.

7. Clinical Chemistry: After 3 months of treatment, SGPT values were increased in female mice at 500 ppm. This was considered to be a treatment-related effect since an increase in MFO activity and an increase in liver weights were observed at this time. Other changes observed at this time were considered to be questionable in terms of their biological significance because they were not seen at later time periods and they were not observed with higher dose levels that were tested in a previous study (mouse subchronic feeding). No treatment-related changes were observed in any of the treated groups at any of the other time periods.

8. Urinalysis: No treatment-related effects were observed in any of the test groups.

9. Hepatic Mixed Function Oxidase Assay: After 3 months of treatment with the test chemical, MFO activity was increased in females at 100 ppm and in both sexes at 500 ppm. At this time point, RH-3866 had no effect on hepatic microsomal protein content at any dose level. After 6 months of treatment, MFO activity was increased in both sexes at 500 ppm. At this dose level, hepatic microsomal protein content was increased 28% and 21% in males and females, respectively. After 12 months of treatment, significant increases in MFO activity were observed in 500 ppm females. Hepatic microsomal protein concentration was not affected at any dose level after 12 months. At this time period, RH-3866 had no effect on peroxisomal ^{14}-palmitoyl-CoA oxidase activity.

10. Gross Pathology: No treatment-related gross changes were observed in any of the treated groups at any time period.

11. Organ Weights: At 3 months, absolute and relative liver weights were significantly increased over controls in both male and female mice fed 500 ppm of the test chemical. No treatment-related changes in organ weights were observed at any dose level at either 6 months, 12 months or 24 months. All the changes that were observed were considered to be either spurious or due to the fact that the control weights were exceptionally low.

12. Histopathology:

    a. Nonneoplastic lesions: Treatment-related changes in the liver were observed in male mice at the 500 ppm level after 3, 6, and 12 months of treatment. These changes included increased incidences and severity of centrilobular hepatocytic hypertrophy,

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Kupffer cell pigmentation, periportal punctate vacuolation and individual hepatocellular necrosis. Following 24 months of treatment, changes in the liver were observed in both sexes at the 500 ppm level. These included increased incidences of focal hepatocellular alterations and multifocal hepatocellular vacuolation. These were not associated with any hypertrophy, hyperplasia or neoplastic proliferations in the liver. No other treatment-related microscopic changes were observed at any dose level. Table I summarizes the liver effects observed.

b. Neoplastic lesions: No treatment-related increases in any neoplasms were observed at any dose level. Hepatocellular hypertrophy and hepatocellular adenomas or carcinomas occurred in mice of all groups, including the controls. RH-3866 had no effect on the severity or incidence of these lesions. Other neoplastic lesions of the liver were also found in all groups: hemangioma, hemangiosarcoma, lymphoreticular lesions, and metastatic or invasive tumors. Adenomas and carcinomas of the lungs were found in all groups as well as lymphosarcomas and other neoplastic lesions of the lymphoreticular system. The attached tables summarize the incidence of neoplastic lesions found. Two tables are given, one for mice which either died or were sacrificed prior to and including 12 months and one for mice which either died or were sacrificed at the termination of the study.

c. Sentinel mice: There was no indication of any intercurrent disease.

13. Quality Assurance Measures: The study was audited and reviewed numerous times by the Quality Assurance Unit for adherence to GLP's and the final report was signed by this group.

C. DISCUSSION: As a chronic feeding study, this appears to be a well conducted study. It is classified as CORE GUIDELINE for a chronic feeding study. The study is classified CORE SUPPLEMENTARY as an oncogenicity study because the Toxicology Branch (TB) does not believe that the top dose level tested was sufficiently high enough. It does not appear that the Maximum Tolerated Dose (MTD) was reached. The effects seen at the highest dose level tested were increases in liver mixed-function oxidase, SGPT and liver weights; and microscopic alterations of the liver consisting of centrilobular hypertrophy, vacuolation, Kupffer cell pigmentation and altered foci (eosinophilic, basophilic, vacuolated and clear cell types). These effects are not considered to be sufficiently severe enough to establish that the highest level tested (500 ppm) approached the MTD.

This chemical was tested in a 3-month dietary subchronic study. The dose levels tested were approximately 3, 10, 30, 100, 300, 1000, 3000 and 10,000 ppm. No effects were observed with dose levels up to and including 300 ppm. At 1000 ppm, increased liver enzyme activity and

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liver weights were observed as well as hepatocytic hypertrophy, vacuolation and a borderline count of individual hepatocytic necrosis. At 3000 ppm, in addition to the effects noted above, an increase in SGOT in males (although not statistically significant) and pigmentation of the Kupffer cells were observed. A more significant count of individual hepatocytic necrosis was seen but this was not noted in any of the animals exposed to 10,000 ppm. At 10,000 ppm, increases in liver enzymes, BUN, and kidney weights were noted in addition to the effects observed above as well as some hematological effects. Clearly, at this dose level more significant toxicological effects were being observed. Based upon the effects noted in the subchronic study in mice, TB believes that higher dose levels should have been used in the mouse chronic study.

Summary of Nonneoplastic Liver Effects Observed in Mice After Chronic Exposure to RH-3866

Observed Effect	Dose Level							
	Males				Females			
	0 ppm	20 ppm	100 ppm	500 ppm	0 ppm	20 ppm	100 ppm	500 ppm
Hepatocellular Centrilobular Hypertrophy								
3 months	1/10	1/10	1/10	9/10				
6 months	2/10	2/10	1/10	9/10				
12 months	5/20	6/20	5/20	16/20				
12-24 months	8/66	6/63	5/65	11/62				
Sentinel (12 mo.)	1/5							
Kupffer Cell Pigmentation								
6 months	0/10	0/10	0/10	5/10				
12 months	4/20	1/20	4/20	12/20				
Sentinel (12 mo.)	2/5							
Periportal Punctate Vacuolation								
3 months	0/10	0/10	0/10	2/10	0/10	0/10	0/10	1/10
6 months	0/10	0/10	0/10	3/10	0/10	0/10	1/10	2/10
12 months (multifocal)	0/20	0/20	0/20	4/20	0/20	1/20	1/20	3/20
Sentinel (multifocal)	0/5	1/5						
Individual Hepatocellular Necrosis								
6 months	1/10	1/10	3/10	3/10				
12 months	2/20	1/20	1/20	6/20	0/20	0/20	1/20	2/20
Sentinel (12 mo.)	2/5	0/5						

Summary of Liver Effects (Continued)

Observed Effect	Dose Level							
	Males				Females			
	0 ppm	20 ppm	100 ppm	500 ppm	0 ppm	20 ppm	100 ppm	500 ppm
Focal Hepatocellular Alterations								
focus/foci, basophilic	2/66	3/63	1/65	4/62	0/64	0/66	1/66	2/67
focus/foci, clear-cell	0/66	0/63	0/65	2/62	0/64	0/66	0/66	0/67
focus/foci, eosinophilic	2/66	1/63	4/65	5/62	2/64	2/66	1/66	4/67
focus/foci, vacuolated cell	0/66	0/63	1/65	0/62	0/64	0/66	0/66	0/67
Total incidence	4/66	4/63	5/6/65*	10/11/62*	2/64	2/66	2/66	6/67
Multifocal Hepatocellular Vacuolation								
centrilobular	1/66	1/63	0/65	1/62	0/64	1/66	0/66	0/67
diffuse	0/66	0/63	0/65	0/62	0/64	0/66	0/66	1/67
multifocal	1/66	0/63	2/65	7/62	3/64	2/66	0/66	7/67

* / = number of mice with hepatocellular alteration/actual incidence of hepatocellular alteration. In 4 instances, a mouse had more than 1 type of hepatocellular alteration (or neoplasia, which were not included in this table).

RIN 1067-98

Myclobutanil Tox. Review

Page_____ is not included in this copy.

Pages 68 through 75 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

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Liver Weights in Males

Dose (ppm) Month	0	20	100	500
3				
Absolute[1]	1.32	1.31	1.37	1.45*
Relative[2]	404	408	406	445*
6				
Absolute	1.82	2.02	1.87	1.95
Relative	482	525	523	537
12				
Absolute	1.86	1.95	1.87	2.01
Relative	480	484	464	527
24				
Absolute	2.44	2.39	2.23	2.37
Relative	0.72	579	572	589

* $p \leq 0.05$; ** $p \leq 0.01$
[1]Absolute expressed in grams
[2]Relative = Organ Wt. X 10000/ Body Weight

Liver Weights in Females

Dose (ppm) Month	0	20	100	500
3				
Absolute[1]	1.16	1.18	1.16	1.30*
Relative[2]	431	420	432	487*
6				
Absolute	1.54	1.62	1.59	1.67
Relative	486	469	470	513
12				
Absolute	1.73	1.64	1.68	1.72
Relative	490	464	496	519
24				
Absolute	1.96	2.20	2.32	2.07
Relative	539	590	642	600

* $p \leq 0.05$; ** $p \leq 0.01$
[1]Absolute expressed in grams
[2]Relative = Organ Wt. X 10000/ Body Weight

SGPT Values in Females (U/L)

Dose (ppm) Month	0	20	100	500
3	19.6	19.6	19.7	31.3**
Std. Dev.	4.9	4.3	3.6	14.1
6	31.9	36.5	29.9	36.6
Std. Dev.	7.4	9.1	6.2	15.5
12	24.4	26.6	23.3	30.3
Std. Dev.	8.3	11.4	7.0	10.3
24	32.4	35.9	41.2	33.0
Std. Dev.	16.6	15.2	22.0	15.6

** $p \leq 0.01$

Mixed Function Oxidase Activity in Males
(per mg Microsomal Protein)

Dose (ppm) Month	0	20	100	500
3	100 ± 22	93 ± 20	107 ± 12	166 ± 19*
6	100 ± 18	114 ± 27	127 ± 12	217 ± 34*
12	100 ± 14	104 ± 39	121 ± 37	136 ± 81

* $p \leq 0.05$

Mixed Function Oxidase Activity in Females
(per mg Microsomal Protein)

Dose (ppm) Month	0	20	100	500
3	100 ± 16	106 ± 18	129 ± 25*	163 ± 15*
6	100 ± 5	96 ± 11	125 ± 24	233 ± 71*
12	100 ± 32	94 ± 29	144 ± 42	324 ± 38*

* $p \leq 0.05$

Mean Body Weights (g) - Males

Dose (ppm) Week	0	20	100	500
0	28.9	28.8	28.8	28.9
4	33.0	33.6	33.4	33.2
8	35.6	36.3	35.4	35.2
12	37.1	38.0	37.1	36.6
24	39.0	39.5	38.2	38.6
52	40.4	41.4	40.2	39.9
78	42.0	42.4	41.9	41.9
104	40.3	40.9	39.4	40.2

Mean Body Weights (g) - Females

Dose (ppm) Week	0	20	100	500
0	23.0	22.8	22.5	23.0
4	26.6	27.3*	26.9	26.1
8	28.7	29.6*	29.1	27.9*
12	30.6	31.4*	30.2	29.7
24	32.8	33.6	32.7	31.4*
52	34.8	35.8	34.8	33.7
78	37.1	37.8	36.5	36.4
104	36.7	37.3	36.7	36.0

* $p \leq 0.05$

Reviewed By: Pamela Hurley, Toxicologist Pamela M. Hurley 4/1/94
Section I, Tox. Branch (7509C)
Secondary Reviewer: Roger L. Gardner, Section Head Roger Gardner 4/1/94
Section I, Tox. Branch (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Oncogenicity Study in Rats (83-2)

SHAUGHNESSY NO./TOX. CHEM. NO.: 128857 / 723K

ACCESSION NO./MRID NO.: 428091-01

DP BARCODE NO.: D193334, D193309, D193312, D193322, D193332

TEST MATERIAL: Myclobutanil

SYNONYMS: Rally®, Nova®, Eagle®

LABORATORY PROJECT I.D.NUMBER(S): HWA 417-471, RH-89RC-260

SPONSOR: Rohm and Haas Company, Spring House, PA 19477

TESTING FACILITY: Hazleton Washington, Inc., Vienna VA 22182

TITLE OF REPORT: RH-3866 Technical (Myclobutanil): 104-Week Dietary Oncogenicity Study in Rats

AUTHOR(S): G. W. Wolfe

REPORT ISSUED: 2/12/93

CONCLUSION: In a two year carcinogenicity study, technical myclobutanil (92.9%) was administered to 50 male and 50 female Sprague-Dawley Crl:CD®BR VAF/Plus® rats at dose levels of 0 or 2500 ppm (125 mg/kg/day) in the diet. An additional 10 animals/sex were added to each group and sacrificed at 52 weeks. A third group of 15 animals/sex were kept on the study without treatment as sentinel animals. These were also sacrificed at 52 weeks. This study was conducted at the request of the HED Carcinogenicity Peer Review Committee because it was determined that the previous study (MRID No. 00165247) was not conducted at sufficiently high dose levels. It was agreed that the rat carcinogenicity study (not the chronic phase) should be repeated in both sexes with the highest dose approaching the "maximum tolerated dose" level (e.g., about 2500 ppm).

At 2500 ppm, statistically significant increases in liver weight (absolute and/or relative) were observed in treated males (20% and 28% higher) and females (0% and 22% higher) at the interim kill, but not at the terminal kill (4.5% and 8.4% higher (♂) and 8.4% and 4.2% higher (♀). Absolute and relative testes weights were significantly lower in treated males at both the interim (63% and 68% of controls for right testes, 58% and 63% of

controls for left testes) and terminal (81% and 85% of controls for right testes and 73% and 74% of controls for left testes) sacrifices. Increases in the incidences of centrilobular to midzonal hepatocellular enlargement and vacuolization were observed in both sexes. These lesions were observed at both 52 weeks and terminal sacrifice. In the testes, an increase in bilateral aspermatogenesis was observed. The decreased spermatogenic activity was associated with an increase in the incidence of hypospermia and cellular debris in the epididymides of the treated males. In addition, an increased incidence of arteritis/periarteritis was noted in the testes of rats which either died on test or were sacrificed in extremis. **In this study, the NOEL could not be established because there were effects at the only dose level tested (decreases in absolute and relative testes weights; increases in the incidences of centrilobular to midzonal hepatocellular enlargement and vacuolization in the liver of both sexes; increases in bilateral aspermatogenesis in the testes; increases in the incidence of hypospermia and cellular debris in the epididymides; and increased incidence of arteritis/periarteritis in the testes).**

Myclobutanil was not oncogenic when tested on Sprague-Dawley rats under the conditions of the study.

The study is classified as Core Guideline when used in conjunction with the previously conducted study (MRID No. 00165247, executive summary below, DER attached). The two studies together satisfy the regulatory requirement for a chronic feeding/oncogenicity study in the rat (83-5).

In the previously conducted study, technical myclobutanil (90.4% and 91.4% pure) was administered to 110 male and 110 female Sprague-Dawley rats in the diet for a period of 24 months. Dietary levels for the low-dose group were 25/35/50 ppm; for the mid-dose group were 100/140/200 ppm and for the high dose group were 400/560/800 ppm. The first dose level in each series was administered for a period of 2 weeks, the second dose level in each series was administered for a period of 2 weeks and the third dose level in each series was administered from weeks 5 to term. The overall calculated mean daily compound consumption was 0, 2.49, 9.84 or 39.21 mg/kg/day for males and 0, 3.23, 12.86 or 52.34 mg/kg/day for females. Of the 110 animals/dose group, ten/sex/dose group were sacrificed at 3 and 6 months, 20/sex/dose group were sacrificed at 12 months and 18 males and 10 females/dose group were sacrificed at 17 months. A sentinel animal program was also conducted separately in which 30 animals/sex were evaluated at 3, 6 and 12 months.

At 9.84 mg/kg/day in males, the mean absolute testes weights were significantly less than controls ($p \leq 0.05$, 77% of controls) at study termination. In addition, an increase in testicular atrophy was observed. In females (12.86 mg/kg/day), liver mixed

function oxidase activity was significantly increased (61% higher) at 3 months, but not after that time. At 39.21 mg/kg/day (52.34 mg/kg/day in females), a statistically significant increase in liver mixed function oxidase activity was observed (34-47% higher) at 3 and 6 months in males and at 3 months in females (78% higher). In females, relative liver weights were significantly increased at 3 months (13% higher) and absolute liver weights were increased at 6 months (20% higher). Statistically significant decreases in absolute testicular weights were observed in males at 12 months (88%). At termination, decreases in absolute and relative testicular weights were also observed (75% and 79% of controls, respectively). In addition, an increase in testicular atrophy was observed. **The LEL is 9.84 mg/kg/day, based on decreases in testes weights and increases in testicular atrophy. The NOEL is 2.49 mg/kg/day.**

The study is classified as Core Guideline when used in conjunction with the present study. The two studies together satisfies the regulatory requirement for a chronic feeding/oncogenicity study in the rat (83-5).

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: 2-Butyl-2-(4-chlorophenyl)-1H-1,2,4-triazole-1-propane-nitrile

Description: Light yellow, crystalline solid
Batch #(s), Other #(s): Lot # 2-2943
Purity: 92.9%
Source: Rohm & Haas
Vehicle: Acetone

2. Test Animals:

Species and Strain (sexes): Male and female Sprague-Dawley Crl:CD®BR VAF/Plus® rats
Age: 4 weeks at receipt (acclimated 9 days prior to randomization; acclimated additional 7 days prior to administration of test diets).
Weight(s): 199.5 - 282.9 g (M), 156.6 - 205.3 g (F) at start of study.
Source(s): Charles River Laboratories, Inc., Raleigh, North Carolina
Housing: Individually in stainless-steel, hanging wire-mesh cages.

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3. Procedure:

a. Dietary Preparation: The test material was placed in a water bath (not exceeding 90°C) for approximately 2-3 hours (until in liquid form) prior to use and stirred to ensure apparent homogeneity. The required amount of test material was then transferred into a heated beaker via plastic syringe and weighed. Approximately 2/3 of the required amount of acetone (5 ml/kg of feed prepared) was added to the test material and stirred for 4-5 minutes. The mixture was then added to a 5-kg feed premix in a blender and the remaining allotment of acetone was used to rinse the beaker. This premix was blended for 30 minutes and then added to the required amount of Purina Rodent Chow and mixed for 1 minute/kg of feed prepared. The control diet was prepared in the same manner, using acetone at an amount equivalent to that used to prepare the test diet.

Frequency of preparation: Every 2 weeks.

Storage conditions: Stored at room temperature.

Stability and Homogeneity Analyses: The report stated that "Four sets (100 g each) of the test and control diet were taken [at] pretest from the top, middle and bottom of the prepared mix and frozen. Three of the sets were sent on dry ice to the Sponsor for homogeneity and Day 0 stability determinations...The fourth set of the test and control diets was sent frozen to the Sponsor after 3 weeks for room temperature stability determinations by the Sponsor." An additional 4 samples were taken as before from the week 55/56 mix. Again, 3 sets were sent frozen to the Sponsor for homogeneity determinations. The additional samples were retained frozen. At both time points, samples of the basal diet were also taken.

Concentration Analyses: Four samples of the test diet and 2 samples from the control and untreated diets were taken from each formulation during weeks 1-10 and frozen. Beginning with the week 11/12 formulation, 7 samples of the test diet and 3 samples of the control and untreated diets were taken and frozen. Samples of those from weeks 1/2, 5/6, 9/10, 13/14, 25/26, 39/40, 51/52, 55/56,

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65/66, 77/78, 91/92, and 103/104 were sent to the Sponsor for analysis.

b. Basis For Selection of Dose Levels: Dietary levels were selected on the basis of the results from a 2-week subacute study and on a 90-day subchronic study in rats. On the basis of these studies and on the basis of a previously submitted chronic feeding/oncogenicity study in rats, the Agency had determined that 2500 ppm was considered to be an appropriate high dose. For this study, the Registrant was only required to test one dose level in both sexes. This study would then be evaluated along with the previous rat study.

c. Animal Assignment and Dose Levels:

Test Group	Dose Administered ppm	Main Study 104 weeks male	Main Study 104 weeks female	Interim Sac. 52 weeks male	Interim Sac. 52 weeks female
1 (control)	0	50	50	10	10
2	2500	50	50	10	10
3 (sentinel)	0	-	-	15	15

d. Additions and/or Alterations in Procedure:

The 15 males and 15 females that were selected for the sentinel program received basal diet only. Otherwise, they were handled in the same manner as the other test animals. Five rats/sex were evaluated for the following serology and parasitology tests prior to administration of the test diets:

Serology: Sendai virus, Mycoplasma pulmonis, Pneumonia virus of mice, Kilham's rat virus, and Sialodacryoadenitis virus.

Parasitology: Radfordia ensifera, Syphacia oblevata and Aspicularis tetraptera.

Pulmonary washings were also conducted. The washes were cultured for isolation of respiratory bacterial pathogens, including corynebacterium kutcheri. In addition, a complete necropsy was conducted and histopathological examinations were conducted on the brain, liver, kidneys, lung, spleen, ileum, colon and any gross lesions. After 1 year, viral serology was conducted on 5 animals/sex. The remaining sentinel animals were bled prior to terminal necropsy; serology tests,

as indicated above, were evaluated, and the animals were necropsied. The organs listed above were preserved but were not microscopically examined. All sentinel animals which died during the course of the study received a complete necropsy and histopathological evaluation.

e. Clinical Observations and Mortality: The animals were observed for clinical signs of toxicity and moribundity twice daily. A careful cageside observation was conducted once daily. A thorough physical examination, including palpation for masses, was conducted weekly through week 16 and biweekly thereafter. Posture, gait, righting reflex and behavior were also evaluated and abnormalities in urine and feces, respiration and body temperature were also noted.

f. Body Weight Determinations: Body weights were measured prior to the start of the study, weekly through week 16 and biweekly thereafter.

g. Food and/or Water Consumption: Food consumption was measured and recorded weekly through week 16 and biweekly thereafter. When obvious spillage or wastage of food was evident, the estimate of food consumption for that animal was excluded from the calculation for that particular time interval. Corresponding feed efficiency and compound consumption values were also calculated.

h. Ophthalmological Examinations: Not conducted.

i. Clinical Pathology: (*) recommended by Guidelines

1) Hematology:

Collection times for blood (including # of animals): Blood samples were collected from all surviving control and treated group animals during weeks 53, 79 and 105 and from all animals sacrificed in extremis. Blood samples were obtained from the tail vein.

The following CHECKED (X) parameters were examined:

	Hematocrit (HCT)*		Mean corpuscular HGB (MCH)
	Hemoglobin (HGB)*		Mean corpuscular HGB conc. (MCHC)
	Leukocyte count (WBC)*		Mean corpuscular volume (MCV)
	Erythrocyte count (RBC)*		Reticulocytes
	Platelet count*	x	Cell morphology
	Total plasma protein (TP)		
x	Leukocyte differential count*		

2) Clinical Chemistry:

Clinical chemistry studies were not conducted.

3) Urinalysis: Urinalysis studies were not conducted.

j. Gross Necropsy:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to end of exposure period and were subjected to complete gross pathological examinations: All animals.

Animals (groups) sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations: All animals.

All surviving animals were weighed on the day of scheduled necropsy and killed by an i.p. injection of sodium pentobarbital and exsanguinated.

The necropsies included examination of the following; all orifices; carcass; cervical tissues and organs; cranial cavity; external surface of the body; external surface of the brain; external surface of the spinal cord and cut surfaces of the brain and spinal cord (at tissue trimming); nasal cavity and paranasal sinuses; and thoracic, abdominal, and pelvic cavities and their viscera. All hollow organs, with the exception of the small intestine, were opened for detection of internal lesions. Bone marrow smears were also collected at the terminal sacrifice.

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k. Histopathology:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to the end of the exposure period and were subjected to microscopic examination: all animals.

Animals (groups) which were sacrificed at the end of the treatment/observation period and were subjected to microscopic examination: All animals.

All preserved tissues were examined using the hematoxylin and eosin stain. Following evaluation, photomicrographs of representative histologic, compound-related alterations of the liver, testes and epididymides, along with normal tissues were taken.

CHECKED (X) tissues were preserved for histopathological examination and (XX) tissues were weighed upon removal from the animal. The (*) tissues were recommended by the Guidelines.

X	
	Digestive system
	Tongue
x	Salivary glands*
x	Esophagus*
x	Stomach*
x	Duodenum*
x	Jejunum*
x	Ileum*
x	Cecum*
x	Colon*
x	Rectum*
xx	Liver*
	Gall bladder*
x	Pancreas*
	Respiratory
x	Trachea*
x	Lung*
x	Larynx
x	Coagulating gland
x	Hardarian gland
x	Zymbal's gland

X	
	Cardiovasc./Hemat.
x	Aorta*
xx	Heart*
x	Bone marrow* (femur & sternum)
x	Lymph nodes*
xx	Spleen*
x	Thymus*
	Urogenital
xx	Kidneys*
x	Urinary bladder
xx	Testes*
xx	Epididymides
x	Prostate
x	Seminal vesicle
xx	Ovaries
x	Uterus* with vagina

X	
	Neurologic
xx	Brain*
x	Periph. nerve*
xx	Spinal cord (3 levels)*
x	Pituitary*
x	Eyes (optic n.)*
	Glandular
xx	Adrenals*
	Lacrimal gland
x	Mammary gland*
xx	Parathyroids*
xx	Thyroids*
	Other
x	Bone* (femur, sternum)
x	Skeletal muscle*
x	Skin
x	All gross lesions and masses

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1. Statistical Analyses: The report stated that "cumulative survival data through Week 105 were analyzed using the National Cancer Institute Package. Trend analysis of survival was evaluated at the 5.0% one-tailed probability level." Mean absolute body weights, body weight changes, total food consumption, leukocyte differentials, terminal body weight and organ weight data were statistically analyzed using a flowchart which included both heterogeneous and homogeneous data. For homogeneous data, an analysis of variance was used. If statistically significant, Dunnett's control versus treatment comparisons were conducted (either for equal variances or for unequal variances if heterogeneous). All parametric comparisons took variance homogeneity/hetergeneity into consideration. If variances of untransformed data were heterogeneous, a series of transformations was performed in an effort to achieve variance homogeneity. When the series of transformations was not successful in achieving variance homogeneity, analyses were performed on rank-transformed data. The following transformations were considered (in order) for heterogeneous data: Log_{10}, Square, Square root, Reciprocal, Angular (Arcsine) or Rank Transformations. All transformations indicated were done on untransformed data.

   The report stated that "tumor incidences were analyzed as follows: increased or decreased neoplastic and nonneoplastic incidences (difference of 2 or more from control) were analyzed by Fisher-Irwin exact test for group comparisons."

B. RESULTS:

1. Dietary Preparation: Results from the stability analyses indicate that the test formulation was stable up to 21 days. The homogeneity studies indicated homogeneous mixing, and the concentration analyses indicated that the formulations averaged 97% of the target dose (the range was 86.9 to 105.0% of the target concentration). The coefficient of variation was 6%. The homogeneity analysis indicated that the % of target concentration ranged from 92.6% to 104% for samples taken at weeks -2 and 55. The stability analysis indicated that the % of target concentration was 96.6% by day 21 (stored at room temperature).

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2. Clinical Observations and Mortality: There were no indications of infectious diseases in the sentinel animals. There was no apparent compound-related effect on survival. Two accidental deaths were noted, one control male sustained a leg injury and a treated female sustained a nasal fracture. The following table summarizes the survival for the control and treated groups.

Survival Data for Male and Female Rats

	Male		Female	
Week	0 ppm	2500 ppm	0 ppm	2500 ppm
0[a]	60/60 (100)	60/60 (100)	60/60 (100)	60/60 (100)
8	60/60 (100)	60/60 (100)	60/60 (100)	59/59 (100)
16	60/60 (100)	60/60 (100)	60/60 (100)	59/59 (100)
24	59/60 (98)	60/60 (100)	59/60 (98)	58/59 (98)
52	57/60 (95)	56/60 (93)	59/60 (98)	58/59 (98)
78	39/50 (78)	35/50 (70)	43/50 (86)	43/49 (88)
90	36/50 (72)	29/50 (58)	33/50 (66)	39/49 (80)
105	17/49 (35)	16/50 (32)	20/50 (40)	28/49 (57)

[a]( ) = % survival from original number in group.

No treatment-related clinical signs of toxicity were observed in the treated groups when compared to the controls. The following table summarizes some of the most common clinical signs that were observed in these animals.

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Summary Incidence of Selected Clinical Signs

	Males		Females	
Observation	0 ppm	2500 ppm	0 ppm	2500 ppm
Hunched	10	7	20	12
Thin	11	8	17	13
Malocclusion	5	10	10	8
Languid	14	11	20	12
Few or No Feces	10	10	16	12
Dyspnea	10	10	19	10
Alopecia - Various Body Areas	15	9	10	5
Sores - Various Body Areas	28	20	15	11
Chromodacryorrhea - Eye(s)	4	8	23	16
Swollen - Various Body Areas	17	9	5	4
Small Moveable Tissue Mass	15	5	47	36
Large Moveable Tissue Mass	10	3	31	19

3. Body Weight Determinations: Statistically significant decreases in mean body weights were observed in the treated group when compared to controls at weeks 26 (96% of controls) and 52 (93% of controls) for males and at week 52 (93% of controls) for females. Statistically significant decreases in body weight gain were observed in the treated group when compared to controls for weeks 0-13, 0-26 and 0-52 for males and for weeks 0-52 for females. Except for weeks 0-52, the mean body weight changes in the treated groups were all above 93% of the control values. For weeks 0-52, the mean body weight changes were 89.9% of the control group for males and 88.2% of the control values for females. These differences between the treated and control groups are not considered to be biologically significant. The following table summarizes body weight gain for both sexes.

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Mean Body Weight Change and Standard Deviations (G)

Dose (ppm)	Week	0-13	0-26	0-52	0-104
		Males			
0	Mean	361.6 ±	461.2 ±	583.1 ±	526.4 ±
	S.D.	51.15	70.52	103.60	110.13
2500	Mean	343.4* ±	431.3* ±	524.3* ±	498.3 ±
	S.D.	37.63	51.96	82.92	131.89
	% Control	94.9	93.5	89.9	94.7
		Females			
0	Mean	141.8 ±	183.7 ±	271.8 ±	275.8 ±
	S.D.	22.62	38.58	73.11	95.03
2500	Mean	134.1 ±	170.3 ±	239.7* ±	294.6 ±
	S.D.	22.07	34.71	70.49	115.93
	% Control	94.6	92.7	88.2	106.8

* Significantly different from control value, $p \leq 0.05$.

4. Food and/or Water Consumption: No treatment-related differences between the treated and control groups were observed. There were no statistical differences at any time point for either sex or for mean total food consumption. In addition, there were no statistically significant differences in the mean efficiency of food utilization at any time point for either sex. The overall mean compound consumption for the treated group was 106.08 ± 31.63 mg/kg/day for males and 135.62 ± 31.67 mg/kg/day for females.

5. Hematology: At week 53, statistically significant decreases in nucleated red blood cells were observed in males. In treated females, the percent of segmented neutrophils were significantly decreased, accompanied by statistically significant increases in lymphocytes at weeks 53 and 105. The same pattern was observed at week 79. However, the differences were not statistically significant. The clinical pathology report stated that "the statistical comparison of percentages of leukocytes has no biologic meaning without knowledge of absolute numbers." It also stated that "the statistical analysis of differences in nucleated erythrocytes (NRBCs) is of questionable value as the presence or absence of NRBCs, rather than the absolute number, is of importance. The cellular morphology was comparable between control and treated groups at weeks 53, 79 and 105. There was no evidence

of an increased incidence of leukemia in the treated animals." The Toxicology Branch agrees with the clinical pathology report. The following table summarizes the data.

Summary of Selected Hematology Data

Parameter	Males - Week			Females - Week		
Dose (ppm)	53	79	105	53	79	105
NRBC - /100 WBC						
0	0.07	0	0	0	0	0
2500	0.00*	0	0	0	0	0
SEG - %						
0	23	30	52	25	31	59
2500	23	31	49	20*	27	46*
LYMPH - %						
0	74	65	43	72	64	37
2500	75	64	46	78*	68	49*

* Significantly different from control value, $p \leq 0.05$.

6. Gross Pathology: Treatment-related effects were observed in the liver and testes in males. Enlarged, thickened lobes were observed in the liver in the unscheduled death animals and small, soft changes were observed in the testes. The latter was especially observed in unscheduled death males. The testicular changes were also seen in the interim and terminal kill animals. There were no treatment-related findings in the females. The following table summarizes the findings.

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Selected Gross Pathology Findings in Males

Observation	0 ppm	2500 ppm
Liver		
Enlarged		
Unscheduled deaths	7/33	21/34
Lobe, thickened		
Unscheduled deaths	0/33	8/34
Kidney		
Enlarged		
Unscheduled deaths	9/33	18/34
Testes		
Small		
Unscheduled deaths		
right	5/33	13/34
left	5/33	15/34
Interim Kill		
right	0/10	5/10
left	0/10	5/10
Terminal Kill		
right	0/17	4/16
left	0/17	5/16
Soft		
Unscheduled deaths		
right	5/33	13/34
left	5/33	13/34
Interim Kill		
right	0/10	4/10
left	0/10	5/10
Terminal Kill		
right	-	-
left	0/17	1/16

7. Organ Weights: A statistically significant increase in mean absolute and relative liver weight was observed in treated males at the interim kill. In females, only the mean relative liver weight was significantly increased at the interim kill. At terminal kill, there were no differences in the liver weights when compared to controls for either sex. The absolute left testis weight and the combined testes weights were significantly lower in the treated males at both the interim and terminal sacrifices. The relative testes weights in the treated animals were significantly less than than the control animals, except for the combined weights at terminal kill. No other treatment-related differences were observed. The following table summarizes the results.

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Selected Organ Weights at Interim and Terminal Sacrifices

Organ	Interim 0 ppm	Interim 2500 ppm	Terminal 0 ppm	Terminal 2500 ppm
		Male		
Liver				
Absolute	19.57	23.48*	19.53	20.41
Relative	2.457	3.136*	2.733	2.963
Testes				
Right				
Absolute	1.87	1.18	1.74	1.41
Relative	0.237	0.161	0.241	0.205
Left				
Absolute	1.85	1.07*	1.71	1.24*
Relative	0.234	0.147*	0.236	0.175*
		Female		
Liver				
Absolute	11.26	11.27	12.86	13.94
Relative	2.478	3.023*	3.065	3.195

*Significantly different from control value, $p \leq 0.05$.

8. Histopathology:

a. Nonneoplastic lesions: Microscopic examination revealed treatment-related lesions in the testes and liver. In addition, compound-related effects were also observed in the epididymides. Statistically significant increases in centrilobular to midzonal hepatocellular enlargement and vacuolization were observed in both sexes. These lesions were observed at both 52 weeks and terminal sacrifice. In the testes, a statistically significant increase in bilateral aspermatogenesis was observed. The decreased spermatogenic activity was associated with a statistically significant increase in the incidence of hypospermia and cellular debris in the epididymides of the treated males. In addition, an increased incidence of arteritis/periarteritis was noted in the testes of rats which either died on test or were sacrificed in extremis. The following tables summarize the findings along with other selected lesions for all animals combined. The second table expands the more significant lesions into levels of severity.

Histopathology Incidence Summary - All Animals

Observation	Males		Females	
Dose (ppm)	0	2500	0	2500
Liver				
No. examined	60	60	60	60
Congestion	18	29*	28	14
Bile duct, fibrosis	28	29	12	24*
Centrilobular to midzonal hepatocellular enlargement	0	52**	0	45**
Centrilobular to midzonal hepatocellular vacuolization	0	32**	0	13**
Testes				
No. examined	60	60	-	-
Hypospermia/Aspermatogenesis, unilateral	3	6	-	-
Hypospermia/ Aspermatogenesis, bilateral	8	30**	-	-
Left Testis				
No. examined	60	60	-	-
Hypospermia	9	7	-	-
Aspermatogenesis	2	27**	-	-
Arteritis/Periarteritis	8	19*	-	-
Right Testis				
No. examined	60	60	-	-
Hypospermia	5	7	-	-
Aspermatogenesis	3	25**	-	-
Arteritis/Periarteritis	9	18*	-	-
Left Epididymis				
No. examined	60	59	-	-
Hypospermia	8	31**	-	-
Lumen, debris, cellular	2	25**	-	-
Perivascular mononuclear infiltration	13	18	-	-
Right Epididymis				
No. examined	59	59	-	-
Hypospermia	6	27**	-	-
Lumen, debris, cellular	2	22**	-	-
Perivascular mononuclear infiltration	11	11	-	-
Left Ovary				
No. examined	-	-	59	60
Follicle, cyst	-	-	10	24**
Right Ovary				
No. examined	-	-	59	60
Follicle, cyst	-	-	12	11

Histopathology Incidence Summary - All Animals

Observation	Males		Females	
Dose (ppm)	0	2500	0	2500
Eye				
No. examined	60	60	60	60
Keratitis, unilateral	3	3	0	4
Keratitis, bilateral	3	2	0	2
Hypopyon	3	2	0	4

* $p \leq 0.05$; ** $p \leq 0.01$

Expanded Histopathology Table for Liver, Testes and Epididymides - All Animals

Observation	Males		Females	
Dose (ppm)	0	2500	0	2500
Liver				
No. examined	60	60	60	60
Centrilobular to midzonal hepatocellular enlargement				
Minimal	0	5	0	2
Slight	0	31	0	38
Moderate	0	16	0	5
Mean of graded findings	0.0	1.9	0.0	1.6
Centrilobular to midzonal hepatocellular vacuolization				
Minimal	0	10	0	1
Slight	0	15	0	2
Moderate	0	7	0	1
Mean of graded findings	0.0	1.0	0.0	0.1
Right Testis				
No. examined	60	60	-	-
Arteritis/Periarteritis				
Minimal	1	3	-	-
Slight	4	8	-	-
Moderate	3	5	-	-
Moderately severe	1	1	-	-
Severe	0	1	-	-
Left Testis				
No. examined	60	60	-	-
Arteritis/Periarteritis				
Minimal	1	3	-	-
Slight	3	8	-	-
Moderate	3	5	-	-
Moderately severe	1	2	-	-
Severe	0	1	-	-

b. Neoplastic lesions: No treatment-related neoplastic lesions were observed. The following table summarizes selected neoplastic lesions of interest.

Selected Neoplastic Lesions

Observation	Males		Females	
Dose (ppm)	0	2500	0	2500
Thyroid				
B-"C" cell adenoma	6/60	8/60	5/60	5/60
Liver				
B-hepatocellular adenoma	2/60	1/60	0/60	0/60
M-hepatocellular carcinoma	1/60	2/60	0/60	0/60
Pancreas				
B-islet cell adenoma	2/60	5/60	3/59	0/60
Right Testes				
B-interstitial cell tumor	1/60	0/60	-	-
Left Ovary				
B-benign granulosa/theca cell tumor	-	-	0/59	1/60
Right Ovary				
N-endometrial sarcoma	-	-	0/59	1/60
Uterus				
B-endometrial stromal polyp	-	-	2/59	3/60
Mammary				
B-fibroadenoma	0/4	1/1	25/60	17/60
M-carcinoma	0/4	0/1	9/60	9/60
Zymbal's Gland				
M-carcinoma	1/58	3/57	0/60	0/60

9. Quality Assurance Measures: Signed Good Laboratory Practice Statement and Quality Assurance Statements were provided.

C. DISCUSSION: This study was conducted at the request of the Toxicology Branch (TB-I) because it was determined that the previous study (MRID No. 00165247) was not conducted at sufficiently high dose levels. On February 9, 1988, the then Toxicology Branch Peer Review Committee met to review the toxicology data base on myclobutanil (see memorandum from J. Quest to L. Rossi, dated 3/1/88). At that meeting, it was agreed that the rat chronic feeding/oncogenicity study should be repeated in both sexes with the highest dose

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approaching the "maximum tolerated dose" level (e.g., about 2500 ppm). This study fulfills that request.

The NOEL cannot be calculated from this study because there were effects at the one dose level tested (2500 ppm or 125 mg/kg/day). Therefore, the NOEL from the previously conducted study will be used: 2.49 mg/kg/day (approximately 50 ppm). The LEL is 9.84 mg/kg/day (approximately 200 ppm) based on testicular atrophy in males. No other significant effects were observed in either sex.

The effects observed in this study were similar to those seen in the previous study. Treatment-related effects were observed in the liver and testes in males. Statistically significant increases in liver weight (absolute and/or relative) was observed in treated males and females at the interim kill, but not at the terminal kill. Testes weights were significantly lower in treated males at both the interim and terminal sacrifices. Increases in the incidences of centrilobular to midzonal hepatocellular enlargement and vacuolization were observed in both sexes. These lesions were observed at both 52 weeks and terminal sacrifice. In the testes, an increase in bilateral aspermatogenesis was observed. The decreased spermatogenic activity was associated with an increase in the incidence of hypospermia and cellular debris in the epididymides of the treated males. In addition, an increased incidence of arteritis/periarteritis was noted in the testes of rats which either died on test or were sacrificed in extremis.

This study, when taken in conjunction with the previously conducted study satisfies the regulatory requirement for a chronic feeding/oncogenicity study in the rat.

Reviewed by: Pamela Hurley P.Hurley 1/11/88
Section 2 , Tox. Branch (TS-769C)
Secondary Reviewer: Edwin Budd
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DATA EVALUATION REPORT

STUDY TYPE: Rat Chronic/Oncogenicity (83-5) TOX. CHEM. NO.: 723K

ACCESSION NUMBER: 266081

TEST MATERIAL: RH-3866

SYNONYMS: Myclobutanil, Systhane, Rally

STUDY NUMBER(S): Sponsor's Project No. 85RC-61, Testing Lab Project No. 8342

SPONSOR: Rohm and Haas Company, Spring House, PA

TESTING FACILITY: Tegeris Laboratories, Inc. Laurel, MD

TITLE OF REPORT: Chronic Toxicity and Oncogenicity Study with RH 3866 in Rats

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IDENTIFYING VOLUME: Volumes 7-13 of 47

CONCLUSION: The NOEL for the study is 2.49 mg/kg/day and the LOEL is 9.84 mg/kg/day based upon testicular atrophy in males. No other significant effects were observed in either sex. The overall mean daily consumption was 0, 2.49, 9.84 and 39.21 mg/kg/day for males and 0, 3.23, 12.86 and 52.34 mg/kg/day for females for the controls, low, mid- and high dose groups, respectively. No oncogenic effects were observed.

Classification: CORE GUIDELINE for the chronic portion of the study and CORE SUPPLEMENTARY for the oncogenicity portion of the study (see discussion).

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: alpha-butyl-alpha-4-chlorophenyl-1-H-1,2,4-triazole-propanenitrile
Description: Solid or viscous solid
Batch #(s), Other #(s): TD #83-260, Lot # LAP 0298 (first 15 weeks); TD #84-038, Lot # 83159-7 (weeks 16ff)
Purity: 90.4% a.i.; 91.4% a.i. (due to error in initial labeling from Sponsor, the dietary conc. of second batch calculated from a value of 92.7% a.i.)
Source: Rohm & Haas

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2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): Male and female Sprague-Dawley rats
Age: 6-8 weeks, dosing to begin after 2-3 week acclimatization period.
Weight(s): Approx. 130-140 grams (F-M, 1 week prior to initiation of study)
Source(s): Charles River Breeding Laboratories, Wilmington, MA

3. Procedure:

a. Dietary Preparation (if applicable): Each jar was heated in a water bath until the sample was liquified; the temperature of water bath did not exceed 90°C. The liquified test chemical was stirred and small aliquots were placed in jars until ready to be used. When used, the sample was heated again, weighed, dissolved in acetone and mixed in the feed. The acetone was evaporated off.
Frequency of preparation: weekly
Storage conditions: room temperature
Stability Analyses: 2-week stability test at all dose levels prior to study
Homogeneity Analyses: Pretest analysis. Samples from top, middle and bottom portions of the feed mixer were retained for analyses. Samples were stored in animal room in feeders and samples obtained after 1 and 2 weeks for assay of compound concentration.
Concentration Analyses: Samples collected at each dose level throughout the study and frozen. Analyses for dose level verification were conducted weekly during first four weeks and subsequently from one set of samples every 4 weeks during the remainder of the study.

b. Basis For Selection of Dosage Levels:

Not stated, however, a subchronic feeding study in rats has been conducted in which the NOEL was 1000 ppm and the LEL was 3000 ppm based upon liver and kidney effects.

c. Animal Assignment and Dose Levels:

Test Group	Dose Administered (ppm) Weeks 1-2	Weeks 3-4	Weeks 5 to term
1-Control	0	0	0
2	25	35	50
3	100	140	200
4	400	560	800

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Number of Animals Sacrificed:

Test Group	Main Study 24 months male	female	Interim Sac. 3 months male	female	Interim Sac. 6 months male	female	Interim Sac. 12 months male	female	Interim Sac. 17 months male	female
1	all surviving		10	10	10	10	20	20	18	10
2	all surviving		10	10	10	10	20	20	18	10
3	all surviving		10	10	10	10	20	20	18	10
4	all surviving		10	10	10	10	20	20	18	10

A sentinel animal program was also used with this study. Thirty male and 30 female rats were used. Prior to the initiation of the study, 5 animals of each sex were subjected to a complete viral and microbiological evaluation. At 3, 6 and 12 months, blood sera and/or live animals were submitted to the diagnostic laboratory for evaluation.

d. Procedures for Studies Other Than Feeding and/or Additions, Changes in Feeding Study: Dietary levels were adjusted on the basis of active ingredient content of the test material. Dietary levels were also adjusted during the initial 5 weeks of the study in order to provide a more nearly equal compound intake, mg/kg/day, during the active growth period of the animals.

e. Clinical Observations and Mortality: Twice daily. Detailed examinations when body weights were measured.

f. Body Weight Determinations: -1 weeks, 0, weekly during first 14 weeks, 1x every two weeks thereafter.

g. Food and/or Water Consumption: -1 weeks, weekly during first 14 weeks, 1x every two weeks thereafter.

h. Ophthalmological Examinations (if applicable): Prior to 12-month and terminal necropsies. Performed on all controls and high dose animals. Will be conducted on other animals if effects noted in high dose animals.

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i. Clinical Pathology: (*) recommended by Guidelines

1) Hematology:

Collection times for blood (including # of animals):
10 males, 10 females/group at 3, 6, 12, 17 months and prior to termination of study.

The following CHECKED (X) parameters were examined:

X		X	
x	Hematocrit (HCT)*	x	Mean corpustular HGB (MCH)
x	Hemoglobin (HGB)*	x	Mean corpustular HGB conc.(MCHC)
x	Leukocyte count (WBC)*	x	Mean corpustular volume (MCV)
x	Erythrocyte count (RBC)*	x	Red cell morphology#
x	Platelet count*		
	Total plasma protein (TP)		
x	Leukocyte differential count*#		

Evaluated only on control and high-dose groups at 6 and 12 months and in all dose groups at 3 months.

2) Clinical Chemistry:

The following CHECKED (X) parameters were examined:

X	Electrolytes:
x	Calcium*
	Chloride*
	Magnesium*
x	Phosphorus*
	Potassium*
	Sodium*

X	Enzymes:
x	Alkaline phosphatase
	Cholinesterase
	Creatinine phosphokinase*
	Lactic acid dehydrogenase
x	Serum alanine aminotransferase (also SGPT)*
x	Serum aspartate aminotransferase (also SGOT)*
x	Gamma glutamyl transpeptidase (GGTP)

X	Other:
x	Albumin*
x	Blood creatinine*
x	Blood urea nitrogen*
x	Cholesterol*
x	Globulins
x	Glucose*
x	Total bilirubin*
x	Total protein*
x	Triglycerides#
x	A/G ratio

12-month and terminal sacrifice only

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3) Urinalysis:

Collection times for urine (including # of animals):
Control and high-dose groups designated for hematology and clinical chemistry collected at 3 (all dose groups), 5, 11, 17 months.

The following CHECKED (X) parameters were examined:

X		X	
x	Appearance*	x	Glucose*
	Volume*	x	Ketones*
x	Specific gravity*	x	Bilirubin*
x	pH	x	Blood*
x	Sediment (microscopic)*		Nitrate
x	Protein*		Urobilinogen

j. Liver Enzyme Assays: Sections of liver from 6 males and 6 females in the control, low, mid and high-dose groups were obtained at the 3, 6 and 12-month sacrifices for determination of mixed function oxidase (MFO) activity. MFO activity was measured by the in vitro enzyme assay of demethylation of aminopyrine (AP). At the 12-month necropsy, livers from 5-6 males and females randomly selected from each group were collected and analyzed for peroxisomal beta-oxidation activity utilizing ^{14}C-palmitoyl-CoA as substrate.

k. Gross Necropsy:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to end of exposure period and were subjected to complete gross pathological examinations:

10/sex/group at 3 and 6 months; 20/sex/group at 12 months; 18 males and 10 females at 17 months. All animals found dead or sacrificed in a moribund condition.

Animals (groups) sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations:

All animals.

l. Histopathology:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to the end of the exposure period and were subjected to microscopic examination:

All animals for liver, testes and ovaries; lungs and kidney: all animals at 12 and 17 months; all organs at 12 months in control and high-dose groups and target organs in mid- and low-dose groups; all organs in animals that were found dead and sacrificed moribund; gross lesions and masses: control and high-dose males and females at 3, 6 and 17 months, all animals at 12 months, all animals that died or were sacrificed moribund.

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Animals (groups) which were sacrificed at the end of the treatment/observation period and were subjected to microscopic examination:

Liver, testes, ovaries, lungs, kidneys, gross lesions and masses in all animals. Otherwise, all tissues required by protocol in control and high-dose groups and target organs in mid and low-dose groups. In addition, other tissues not required by protocol occasionally were inadvertently examined in some males and females at the 12 and 17-month interim and terminal sacrifices.

CHECKED (X) tissues were preserved for histopathological examination and (XX) tissues were weighed upon removal from the animal. The (*) tissues were recommended by the Guidelines.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue		Aorta*	xx	Brain*
x	Salivary glands*	xx	Heart*	x	Periph. nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)*
x	Stomach*	x	Lymph nodes*	x	Pituitary*
x	Duodenum*	xx	Spleen*	x	Eyes (optic n.)*
x	Jejunum*	x	Thymus*		Glandular
x	Ileum*		Urogenital	xx	Adrenals*
x	Cecum*	xx	Kidneys*		Lacrimal gland
x	Colon*	x	Urinary bladder*	x	Mammary gland*
x	Rectum*	xx	Testes*	x	Parathyroids*
xx	Liver*	x	Epididymides	x	Thyroids*
	Gall bladder*	x	Prostate		Other
x	Pancreas*	x	Seminal vesicle	x	Bone*
	Respiratory	xx	Ovaries	x	Skeletal muscle*
x	Trachea*	x	Uterus*	x	Skin
x	Lung*			x	All gross lesions and masses
x	Larynx				

m. Statistical Analyses: one-way Analysis of Variance followed by Dunnett's t-test. Percent survival estimated with Lifetest Procedure (SAS Institute).

B. RESULTS:

1. Dietary Preparation: Measured dose levels ranged between 83-108% of the desired levels. The average was 95%.

2. Clinical Observations and Mortality: Treatment with the test chemical did not affect the survival of males or females at any dose level. No difference in mortality was noted. By week 105 the total mortalities in males were 35, 35, 32 and 30 in the control, low, mid and high-dose groups, respectively, and 37, 39, 40, and 35 in females respectively. There were no clinical signs observed

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that appeared to be related to treatment. The most common clinical signs that were observed throughout the study in all groups, including controls were dermal alopecia, rough haircoat, rash, footpad swelling and mechanical injuries.

3. Body Weight Determinations: The mean weekly body weights of the treated males were similar to controls during the first 8 weeks of the study. After that time, the mean weekly body weights of the high dose males began to decline relative to the control values and by week 22, they were significantly less than controls. This continued up to week 40. Although the body weights of the high dose males were statistically significantly less than the controls, the values still remained within 95-97% of the control values. After that time, the mean body weights of the high dose males were always less than controls, but were only significantly less at weeks 56, 80, 82 and 84. The data suggest that the test chemical induced a decrease in the mean body weights of the males in the high dose group when compared to controls between 6 and 18 months. The body weights at the lower dose levels were generally slightly lower than controls during this time period, but the lower values were not considered to be biologically significant. For females, the test chemical appeared to have no effect on the body weights of the treated animals during the first year of the study. During the second year, the test chemical appeared to have a marginal effect on the body weights of the high dose females relative to controls. The body weights were generally lower than controls during weeks 54 to 96 and the differences were statistically significant at weeks 66-72, 76-84 and 92. During weeks 76-84, the body weights were generally between 88-90% of the control values.

4. Food and/or Water Consumption: For males, food consumption was generally lower in the high dose animals when compared to controls, starting around the fifth week. Food consumption was statistically significantly lower for fifteen weeks within the fifth to seventy-eighth week; however, the values never dropped below 91% of the control values. Beginning at week 80, food consumption was similar to controls. No significant differences were noted for the two lower dose male groups. For females, in general, no significant changes in food consumption were observed between the treated and control animals. Based upon the food consumption values, the overall mean daily compound consumption for males was 0, 2.49, 9.84 and 39.21 mg/kg/day and for females was 0, 3.23, 12.86 and 52.34 mg/kg/day for controls, low, mid- and high dose groups, respectively.

5. Ophthalmological Examinations: There were no indications of compound-related ocular abnormalities. The most prevalent abnormality observed prior to termination of the study was diagnosed as conjunctivitis secondary to infectious diseases or dental abnormality.

6. Hematology: No treatment-related differences were observed in any of the treated groups when compared to controls.

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7. Clinical Chemistry: There were a number of occurrences of statistically significant differences in several clinical chemistry values. However, for various reasons, each one of these were considered to be biologically insignificant. Therefore, there were no treatment-related changes observed in any of the clinical chemistry parameters.

8. Urinalysis: No consistent differences were noted between the treated and control groups.

9. Hepatic Enzyme Assays: A slight increase in hepatic mixed function oxidase (MFO) activity (34-47%) was seen in high-dose level males at 3, 6 and 12 months. The increases were statistically significant at 3 and 6 months. MFO activity was significantly increased in females in both mid- and high dose groups (61% and 78%, respectively) at 3 months. At 6 and 12 months, slight increases in high dose females were observed but were not statistically significant. RH-3866 had no effect on hepatic peroxisomal ^{14}C-palmitoyl-CoA oxidase activity in rats at dose levels up to 800 ppm after 12 months of dietary treatment.

10. Gross Pathology: The distributions and incidences of palpable masses in both males and females, as well as the mean "time-to-tumor" for the masses indicate that the presence of the masses were unrelated to treatment. The number of animals in each dose group with palpable masses was 1, 7, 2 and 4 in males for the first 12 months; 15, 13, 16 and 9 in males for months 13 to termination; 7, 9, 13 and 2 in females for the first 12 months; and 47, 46, 38 and 39 in females for months 13 to termination in control, low, mid- and high-dose groups respectively.

    All gross lesions observed at 3 and 6 months were considered to be unrelated to treatment. This was also true for the gross lesions observed at 12 months except for testicular lesions. Testicular reduction in size was seen in 0, 1, 1 and 3 animals in the control, low, mid and high dose animals, respectively. The higher incidence observed in the high dose animals correlates with the reduction in testicular weights in this dose group. At 17 months, again, only the testicular effects were considered to be related to treatment with the test chemical. Bilateral reduction in testicular size was observed in 2, 2, 0 and 6 animals in the control, low, mid and high dose groups, respectively. At terminal sacrifice, with the exception of the testicular effects, all observed lesions appeared to be unrelated to treatment. Decrease in testicular size was seen in 0, 2, 7 and 6 males respectively, in controls, low, mid- and high dose animals. In addition, a second testicular lesion characterized as "soft" was also seen in 1, 1, 5, and 5 males in the controls, low, mid- and high dose groups, respectively. Reduction in testicular size was also seen in the treated animals that died prior to the completion of the study (2, 6, 2 and 7 in controls, low, mid- and high dose animals, respectively).

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11. Organ Weights: In males, no significant differences were observed between mean liver weights and between mean liver-to-body weight ratios in the treated animals versus the controls at any of the sacrifice times. In females, the mean liver-to body-weight ratio in the high dose animals was significantly higher than controls at 3 months (113%) and the mean liver weights in the high dose animals were significantly higher than controls at 6 months (120%). At other times there were increases in liver weights and liver-to-body weight ratios in one or more of the treated groups, but none were statistically significant. The authors state that these results suggest that there may be a marginal effect of the chemical on mean hepatic weights in female rats.

At 12 months, the mean testicular weights and testes-to-body weight ratios in all groups of the treated animals were lower than controls, but only the mean testicular weights in the high dose animals were statistically significant (88% of controls). At 17 months, none of the means for the treated animals were statistically significant, but the means were slightly lower than controls for high dose males (88% and 90% for testes and testes-to-body weight ratio, respectively). At termination, the mean testicular weights of both mid- and high dose groups (77% and 75% of controls, respectively) and the mean testes-to-brain weights of the high dose group were significantly lower than controls (79% of controls). The results suggest an effect at the high dose for testicular weights and a possible marginal effect at the mid-dose. No effects were observed in any of the other organ weights. The changes observed were considered to be random occurrences.

12. Histopathology:

a. Nonneoplastic lesions: With the exception of testicular lesions, all nonneoplastic microscopic findings in the study were considered to be incidental to treatment. Incidental lesions were normally found in the liver, kidneys, lung, heart, spleen, adrenals, pancreas, and thyroid gland. The incidences of unilateral and bilateral testicular atrophy are summarized in Table I. The incidence was similar between control and low dose animals, but was increased in mid- and high dose animals. Microscopically, the seminiferous tubules were frequently devoid of spermatid formation and germinal epithelial cells. In several cases, only Sertoli cells remained. In addition to atrophy, microscopic findings included polyarteritis, periarteritis, mineralization of arterioles and occasionally a sperm granuloma, an interstitial cell tumor, scrotal varicocele, orchitis, oligospermatogenesis and bilateral seminiferous tubule atrophy.

b. Neoplastic lesions: No neoplastic lesions were observed that were considered to be related to treatment. Neoplastic lesions were generally observed either in low incidence in all groups, including controls or only in an occasional animal. Tumors that were seen included hepatocellular adenomas; pituitary adenomas

and chromophobe adenomas; mammary gland adenomas, adenocarcinomas and fibroadenomas and islet cell adenomas of the pancreas (see Table II).

13. Quality Assurance Measures: Quality assurance measures were followed and the study was audited on a monthly basis. The report was signed by the Quality Assurance Manager.

C. DISCUSSION: There were several places in which the procedures in this study deviated from the EPA Testing Guidelines: 1) For the clinical chemistry studies, the same animals were not used for each time point. These animals were sacrificed at each time point. 2) Histopathology on gross lesions in the low and mid-dose animals sacrificed at 3, 6 and 17 months was not conducted. Microscopic examinations were conducted on gross lesions in these dose groups at the 12 month sacrifice, at termination and on all animals that either died or were sacrificed in extremis during the study. 3) The lungs and the kidneys were not examined in the low and mid-dose groups at the 3 and 6 month sacrifice times.

Point number 1 is not considered to be one that would significantly affect the outcome of the study. Since microscopic examinations were conducted on gross lesions and the specific organs mentioned above in point 3 from both the low and mid-dose groups at other sacrifice times, and since the results were negative except for testicular effects, these points are also not considered to be significant to the outcome of this study. Therefore, this study is classified as CORE GUIDELINE for the chronic portion of the study. The NOEL for the study is 2.49 mg/kg/day based upon testicular atrophy in males and the LOEL is 9.84 mg/kg/day. In females, a significant increase in hepatic mixed function oxidase activity was observed in both the mid- and high dose groups at 3 months, the mean liver-to body-weight ratio was elevated at 3 months and the mean liver weight was elevated at 6 months. These effects are most likely an adaptive response. Therefore, it appears that the chemical has a minimal effect on females at the dose levels tested.

The chemical was not oncogenic under the conditions of the study. The study is classified as CORE SUPPLEMENTARY for the oncogenic portion of the study because the Toxicology Branch (TB) does not believe that the top dose level tested was sufficiently high enough. It does not appear that the Maximum Tolerated Dose (MTD) was reached. Other than testicular atrophy, there was a marginal effect on liver weights in females at the highest dose level tested. Body weights were marginally decreased in males, but food consumption was also less than controls. In addition, an increase in liver mixed function oxidase was also observed. These effects are not considered severe enough to establish that the highest dose level tested (800 ppm) approached the MTD. Testicular atrophy is not likely to be life-threatening, and thus, higher dose levels could have been used. In addition, this lesion did not appear in the rat subchronic study. The dose levels selected for the chronic study appeared to be selected on the basis of increase in liver weights and liver enzyme induction.

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The dose levels selected for the rat subchronic study were approximately 10, 30, 100, 300, 1000, 3000, 10,000 and 30,000 ppm. No effects were observed with dose levels up to and including 100 ppm. At 300 ppm, increases in liver mixed function oxidase activity were observed. At 1000 ppm, increases in the mean relative liver weights in females and accentuated liver architecture (seen grossly but nothing was noted in the microscopic examinations) were observed in addition to what was seen at 300 ppm. At 3000 ppm, increases in kidney and liver weights and SGOT were observed as well as that noted for 1000 ppm. In addition, hepatocellular hypertrophy was seen in the majority of the animals and hepatocellular necrosis was seen in 1/10 males and 3/10 females. Pigmentation of the convoluted tubular epithelium of the kidney was also observed at this dose level in males only. At 10,000 ppm, the effects noted at 3,000 ppm were observed (hepatocellular necrosis was seen in only 1 animal of each sex) as well as vacuolated swollen hepatocytes, blood effects (indications of red cell destruction and compensatory red cell production and hemosiderosis), increases in liver enzymes and BUN, Kupffer cell pigmentation. All of the animals which received 30,000 ppm died prior to the end of the testing period. Based upon the effects noted in the subchronic study in rats, TB believes that higher dose levels should have been used in the rat chronic/oncogenicity study.

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TABLE I
INCIDENCE OF UNILATERAL AND BILATERAL TESTICULAR ATROPHY

	Control	Low	Mid	High
12-Month Sacrifice				
Bilateral	0/20	0/19	1/20	3/20
Unilateral	0/20	1/19	0/20	0/20
17-Month Sacrifice				
Bilateral	2/18	2/18	0/18	4/18
Unilateral	2/18	2/18	0/18	1/18
Terminal Sacrifice				
Bilateral	2/17	1/19	5/20	12/22
Unilateral	2/17	3/19	6/20	2/22
Animals That Died or Were Sacrificed Moribund				
Bilateral	1/35	4/35	10/32	12/30
Unilateral	6/35	4/35	5/32	5/30
Total Incidence of Testicular Atrophy Across All Groups*				
Bilateral	5/110	7/110	16/110	31/110
Unilateral	10/110	10/110	11/110	8/110

* Including 3 and 6 month sacrifices (10 animals apiece, except low dose at 3 months had only 9 animals).

Table II. Incidence of Neoplastic Microscopic Findings (Summary of Significant Lesions)

Organ	Males				Females			
(Lesion)	Control	Low	Mid	High	Control	Low	Mid	High
				12-Month Sacrifice				
Liver								
Hepatocellular Adenoma	0/20	0/19	0/20	0/20	0/19	0/20	0/20	2/20
Pituitary								
Adenoma	0/20	1/18	0/20	0/19	4/19	3/20	3/20	3/20
Mammary Gland								
Adenocarcinoma	0/1	0/1	0/0	0/4	4/10	2/3	2/5	2/17
				17-Month Sacrifice				
Pituitary								
Chromophobe Adenoma	5/6	0/0	0/0	3/3	2/3	0/0	0/0	0/0
Adenoma	1/6	0/0	0/0	0/3	1/3	0/0	0/0	0/0
Mammary Gland								
Adenoma	0/1	0/0	0/0	0/0	2/5	0/0	0/0	1/3
				Terminal Sacrifice				
Liver								
Hepatocellular Adenoma	0/17	1/19	0/19	0/22	0/24	0/20	0/20	1/25
Pancreas								
Islet Cell Adenoma	3/17	0/5	0/3	2/22	1/24	1/13	0/13	1/25
Pituitary								
Chromophobe Adenoma	12/17	1/5	3/4	7/22	14/23	18/19	10/15	18/25
Mammary Gland								
Adenocarcinoma	0/2	0/1	0/0	0/3	1/23	0/15	1/11	0/25
Fibroadenoma	0/2	1/1	0/0	0/3	10/23	11/15	6/11	11/25
Adenoma	0/2	0/1	0/0	0/3	3/23	6/15	4/11	2/25

Mean Body Weight Data for Males (g) [% of Control]

Dose (mg/kg/day) Week	0	2.49	9.84	39.21
0	190.2	187.1 [98.3]	187.8 [98.7]	188.3 [99.0]
4	356.2	355.5 [99.8]	352.2 [99.9]	348.3 [97.8]
12	507.9	509.3 [100]	507.7 [100]	499.5 [98.3]
24	618.1	622.0 [100.6]	611.1 [99.9]	597.7* [96.7]
36	702.1	692.5 [98.6]	687.0 [97.8]	665.1* [94.7]
52	778.3	763.3 [98.1]	763.7 [98.1]	746.0 [95.8]
78	834.2	787.9 [94.4]	812.1 [97.4]	778.9 [93.4]
104	667.3	672.0 [100.7]	642.0 [96.2]	657.3 [98.5]

* $p \leq 0.05$

Mean Body Weight Data for Females (g) [% of Control]

Dose (mg/kg/day) Week	0	3.23	12.86	52.34
0	163.9	164.1 [100.1]	162.2 [99.0]	163.1 [99.5]
4	236.7	233.1 [98.5]	233.3 [98.6]	233.1 [98.5]
12	293.6	296.0 [100.8]	290.0 [98.8]	289.7 [98.7]
24	340.9	345.2 [101.3]	336.8 [98.8]	343.8 [100.9]
36	382.7	390.8 [102.1]	377.2 [98.6]	379.4 [99.1]
52	443.6	455.8 [102.8]	436.3 [98.4]	439.2 [99.0]
78	520.8	507.7 [97.5]	512.1 [98.3]	458.5* [88.0]
104	446.8	444.3 [99.4]	434.0 [97.1]	444.5 [99.5]

* $p \leq 0.05$

Mean Food Consumption Data for Males (g/Rat/Week) [% Control]

Dose (mg/kg/day) Week	0	2.49	9.84	39.21
1	182.6	179.7 [98.4]	181.0 [99.1]	179.5 [98.3]
4	193.0	198.6 [102.9]	197.6 [102.3]	194.6 [100.9]
12	222.3	219.6 [98.8]	219.2 [98.6]	215.4 [96.9]
24	212.8	212.1 [99.7]	211.5 [99.4]	209.5 [98.4]
36	209.9	209.2 [99.7]	207.0 [98.6]	201.1 [95.8]
52	229.0	224.6 [98.0]	227.5 [99.3]	219.1 [95.7]
78	229.9	197.9* [86.1]	237.7 [103.4]	209.2* [91.0]
104	210.7	242.4 [115.0]	199.3 [94.6]	221.8 [105.3]

* $p \leq 0.05$

Mean Food Consumption Data for Females (g/Rat/Week) [% Control]

Dose (mg/kg/day) Week	0	3.23	12.86	52.34
1	150.7	145.1 [96.3]	142.1* [94.3]	144.8* [96.1]
4	163.4	161.4 [98.8]	160.1 [98.0]	159.8 [97.8]
12	152.5	148.9 [97.6]	153.8 [100.9]	155.4 [101.9]
24	168.7	172.3 [102.1]	170.8 [101.2]	167.7 [99.4]
36	164.0	161.5 [98.5]	157.8 [96.2]	162.3 [99.0]
52	179.7	179.9 [100.1]	172.9 [96.2]	174.5 [97.1]
78	197.04	197.22 [100.1]	192.0 [97.4]	182.0 [92.4]
104	177.3	179.6 [101.3]	161.1 [90.9]	161.0 [90.8]

* $p \leq 0.05$

Hepatic Mixed Function Oxidase (nmole/g Liver/min)

Month	3	6	12
	Male		
Dose (mg/kg/day)			
0	100 ± 17	100 ± 21	100 ± 26
2.49	96 ± 29	93 ± 27	116 ± 24
9.84	119 ± 26	101 ± 25	123 ± 39
39.21	147 ± 30*	145 ± 25*	134 ± 31
	Female		
Dose (mg/kg/day)			
0	100 ± 26	100 ± 15	100 ± 37
3.23	125 ± 29	91 ± 29	117 ± 30
12.86	161 ± 35*	91 ± 23	99 ± 33
52.34	178 ± 20*	133 ± 11	140 ± 59

* $p \leq 0.05$

Selected Absolute (g) and Relative Organ Weights in High Dose Animals (control/high dose)

	Male		Female	
Organ Month	Absolute	Relative	Absolute	Relative
Liver				
3	16.4/17.7	3.2/3.6	9.3/10.2	3.4/3.9*
6	19.8/21.9	3.3/3.8	10.9/13.1*	3.3/3.6
12	25.7/25.5	3.8/3.8	14.5/16.2	3.7/3.7
17	28.2/31.3	3.6/3.9	16.1/17.0	3.9/3.8
Terminal	26.5/25.2	4.0/3.9	17.4/19.3	4.0/4.5
Testes				
3	-	-	-	-
6	-	-	-	-
12	3.8/3.3	0.56/0.51	-	-
17	3.4/3.0	0.43/0.39	-	-
Terminal	3.2/2.4*	0.44/0.388	-	-

* $p \leq 0.05$

Reviewed By: Pamela Hurley, Toxicologist Pamela M. Hurley 4/8/94
Section I, Tox. Branch (7509C)
Secondary Reviewer: Roger L. Gardner, Head Roger Gardner 4/13/94
Section I, Tox. Branch (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Developmental Toxicity 83-3 (b) - **Supplemental DER**

SPECIES: Rabbit

SHAUGHNESSY NO./CASWELL NO.: 128857 / 723K

ACCESSION NUMBER/MRID NO.: 00164971

TEST MATERIAL: RH-53,866

SYNONYMS: Myclobutanil, Rally, Systhane, Nova

REPORT NUMBER: 83R-217

SPONSOR: Rohm and Haas Company, Philadelphia, PA

TESTING FACILITY: Rohm and Haas Company, Toxicology Dept., Spring House, PA 19477

TITLE OF REPORT: Teratology Study with RH-53,866 in Rabbits

AUTHOR(S): R. D. Costlow and W. W. Kane

REPORT ISSUED: 11/15/84

CONCLUSION: RH-53,866 (technical myclobutanil, 90.4%) was tested in a developmental toxicity study in rabbits. Five groups of 18 female New Zealand White rabbits from Hazleton Dutchland, Denver, PA, received 0 (water control), 0 (Hi-Sil control), 20.0, 60.0 or 200.0 mg/kg/day a.i. mycobutanil by oral gavage (5 ml/kg b.w.) on days 7 through 19 of gestation. The test material was prepared with the solid adsorbent (carrier) Hi-Sil 233 and with 1.0% methylcellulose (vehicle). The females were injected i.v. with chorionic gonadotropin and artificially inseminated 3 hours later. The day on which insemination occurred was considered to be day 0 of gestation.

No maternally toxic effects were observed at either 20 or 60 mg/kg/day. At 200 mg/kg/day, decreases in maternal body weight and body weight gain during the dosing period (0.03 and -0.02 kg in the controls versus -0.28 kg in the treated group) with a rebound during the post-dosing period, irregular feces, bloody urine, bloody urogenital or anal area and blood and/or aborted material were observed in the drop pan. Three does aborted their litters, however, 2 of those litters were totally resorbed (early). There were no effects noted at necropsy. **The maternal toxicity LOEL = 200 mg/kg/day and the maternal toxicity NOEL = 60**

mg/kg/day based on reduced body weight and body weight gain during the dosing period, clinical signs of toxicity and possibly abortions.

No developmentally toxic effects were observed at either 20 or 60 mg/kg/day. At 200 mg/kg/day, there were increases in resorptions, resorptions/litter, decreases in litter size ($p < 0.05$) and a decrease in the viability index ($p < 0.05$). **The LOEL for developmental toxicity is 200 mg/kg/day and the NOEL for developmental toxicity is 60 mg/kg/day based on increases in resorptions, decreases in litter size and a decrease in the viability index.**

The study is classified as Core Minimum data (acceptable) and satisfies the requirement (83-3 b) for a developmental toxicity study in rabbits.

This is a supplemental DER which includes the extra tables needed for a complete analysis. The maternal NOEL and LEL supercede the previous NOEL and LEL.

RESULTS:

Summary of Maternal Clinical Signs[a]

Dose (mg/kg/day) Observation	0 (water)	0 (vehicle)	20	60	200
	Days 0 - 7 of Gestation				
Irregular feces	1(3)[b]	2(3)	4(11)	0(0)	1(2)
Bloody urine	-	-	-	-	-
Bloody urogenital or anal area	-	-	-	-	-
Blood and/or aborted material in pan	-	-	-	-	-
	Days 8 - 29				
Irregular feces	9(28)	8(45)	6(59)	10(49)	16(112)
Bloody urine	0(0)	0(0)	1(1)	1(3)	8(35)
Bloody urogenital or anal area	0(0)	0(0)	0(0)	0(0)	2(4)
Blood in pan	0(0)	0(0)	1(1)	1(4)	7(22)
Aborted material in pan	0(0)	0(0)	0(0)	1(1)	3(3)

[a]Includes non-pregnant animals. [b]Data presented as # animals (total occurrences).

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Summary of Maternal Weight During Gestation[a,b,c,e]

	Dose (mg/kg/day) Day 7 to 19 of Gestation				
	(Water Control) 0.0	(HiSil Control) 0.0	20.0	60.0	200.0
# Inseminated	18	18	18	18	18
# Pregnant	13	12	14	15	14
Fertility index[d]	0.72	0.67	0.78	0.83	0.78
# Died (died pregnant)	0	0	0	0	2 (1)
# Aborted	0	0	1	1	3
Maternal Weight					
Day 0	3.66	3.84	3.84	3.81	3.79
Day 4	3.72	3.91	3.90	3.86	3.81
Day 7	3.77	3.93	3.89	3.85	3.82
Day 11	3.76	3.89	3.86	3.77*	3.64*
Day 15	3.83	3.90	3.89	3.81	3.57*
Day 20	3.80	3.91	3.89	3.79	3.67*
Day 25	3.91	4.01	4.02	3.90	3.85*
Day 29	3.98	4.10	4.06	3.98	3.89*

[a]Data are presented as Mean (N).
[b]Maternal weights are in kilograms
[c]Statistical comparisons were based on analysis of mean weights adjusted for the weight on Day 7 of gestation; controls were combined.
[d]Fertility index = # pregnant/# inseminated
[e]Mean values include only pregnant females; aborted litters included for all days prior to abortion.
* Statistically significant from combined controls at $p < 0.05$.

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Body Weight Gains (Kg)[a,b]							
Days	0-7[d]	7-11[d]	11-15[d]	15-20[d]	7-20	20-29[d]	0-29
Dose (mg/kg/day)							
Water Control	0.11	-0.01	0.07	-0.03	0.03 0.04[c]	0.18	0.33 0.03
Vehicle Control	0.09	-0.04	0.01	0.01	-0.02 0.05	0.19	0.26 0.05
20	0.05	-0.03	0.03	0.00	0.04 0.03	0.17	0.26 0.04
60	0.04	-0.08	0.04	-0.02	-0.06 0.05	0.19	0.18 0.05
200	0.03	-0.18	-0.07	0.10	-0.28* 0.04	0.22	-0.02 0.05

[a]Data extracted from report number 83R-217, table 2.
[b]Corrected body weight gain for entire gestation period not recorded because uterine weights were not recorded.
[c]SEM
[d]Calculated by Agency reviewer from mean body weight data.

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Cesarian Section Observations

Dose (mg/kg/day)	0 (water)	0 (vehicle)	20	60	200
# Animals Assigned	18	18	18	18	18
# Animals Inseminated	18	18	18	18	18
# Pregnant	13	12	14	15	14
Fertility index[a]	0.72	0.67	0.78	0.83	0.78
Maternal Wastage					
# Died	0	0	0	0	2[b]
# # Died/Pregnant	0	0	0	0	1
# Non pregnant	5	6	4	3	4
# Aborted	0	0	1	1	1[h]
# Premature Delivery	0	0	0	0	0
Corpora Lutea/Dam	11.31	12.75	11.00	11.21	7.56[c]
Implantations/Dam	6.92	7.42	7.92	6.64	7.44[c]
Live Fetuses/Dam[e]	6.31	6.75	7.38	6.50	2.70*
# Litters with fetuses	13	12	13	14	3
Total Resorptions	8	8	7	9	11
Early	7	4	3	8	9
Late	1	4	4	1	2
Resorptions/Dam	0.62	0.67	0.54	0.14	4.90
Dead Fetuses/Dam	0.0	0.0	0.0	0.0	0.0
Mean Fetal Weight (g)	46.50	45.42	42.95	45.34	37.47
Implantation Index[d,e]	0.61	0.58	0.72	0.59	0.98*[c]
Fetal Sex Ratio[g]	1.48	1.03	0.92	1.17	2.38
Viability Index[e,f]	0.91	0.91	0.93	0.98	0.35*
Litters w/ Resorptions	1	4	5	3	14[i]
Litters w/ 2 Resorptions	0	1	2	0	1
Litters w/ 3 or more Resorptions	1	1	0	1	1
Litters Totally Resorbed	0	0	1[i]	0	10[i]

[a]Fertility index = # pregnant/# inseminated.
[b]Two died in the high dose group due to intubation errors.
[c]One animal excluded from calculations because the corpora lutea were not observed correctly.
[d]Implantation index = # implantation sites/# corpora lutea
*Statistically significant from combined controls ($p < 0.05$).
[e]Statistically analyzed. All others not analyzed.
[f]Viability index = # live fetuses/# implantations.
[g]Fetal sex ratio = # male fetuses/# female fetuses
[h]The text stated that there were 3 abortions. However, 2 of those abortions had total resorbed litters (early resorptions). Therefore, they were counted as totally resorbed litters.
[i]Includes litters listed as aborted but totally resorbed early (1 at low dose, 2 at high dose) and 1 doe which died due to intubation error on day 19 (high dose).

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DISCUSSION:

Based on the data from these tables, the following corrections need to be made for the first DER.

Maternal Toxicity

1. From the body weight and body weight gain data, it does not appear that there was an effect on body weight and body weight gains at 60 mg/kg/day. Therefore, the LOEL for maternal toxicity is raised from 60 mg/kg/day to 200 mg/kg/day and the NOEL is raised from 20 mg/kg/day to 60 mg/kg/day.

2. In the paragraph on maternal observations, there were statements concerning the conceptuses from the 2 aborted litters, one each in the 20 and the 60 mg/kg/day dose groups. For the 20 mg/kg/day group, the statement is correct in that all of the conceptuses were early resorptions. For the 60 mg/kg/day group, only 7 of 10 conceptuses were resorped early. The other 3 were aborted.

Developmental Toxicity

1. In the paragraph on ovarian, uterine and fetal data, there was a statement that in the 20 and 60 mg/kg/day groups, there were no litters with more than 2 resorptions and no litters completely resorbed. There was also a statement that at 200 mg/kg/day, there was an increased incidence of the number of litters with more than 2 resorptions. In examining the individual animal data, at 20 mg/kg/day, there was 1 litter which was totally resorbed (early resorptions). This litter had been counted as an abortion and was not originally considered. At 60 mg/kg/day, there was 1 litter with 3 or more resorptions. At 200 mg/kg/day, the statement that there was an increased incidence of the number of litters with more than 2 resorptions is inaccurate because most of the litters were totally resorbed and should not be included.

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Reviewed by: Pamela Hurley
Section 2 , Tox. Branch (TS-769C)
Secondary Reviewer: Edwin Budd
Section 2 , Tox. Branch (TS-769C)

Budd 1/12/88

DATA EVALUATION REPORT

STUDY TYPE: Teratogenicity (83-3) - Rabbit TOX. CHEM. NO.: 723K

ACCESSION NUMBER: 266098

TEST MATERIAL: RH-53,866

SYNONYMS: Systhane, Rally, Nova

REPORT NUMBER: 83R-217

SPONSOR: Rohm and Haas Company, Philadelphia, PA

TESTING FACILITY: Rohm and Haas Company, Toxicology Dept., Spring House, PA 19477

TITLE OF REPORT: Teratology Study with RH-53,866 in Rabbits

AUTHOR(S): R.D. Costlow and W.W. Kane

REPORT ISSUED: November 15, 1984

IDENTIFYING VOLUME: Volume 24 of 47

CONCLUSION: RH-53,866 was not teratogenic at any dose level up to 200.0 mg/kg/day, the highest dose level tested. The NOEL for maternal toxicity was 20.0 mg/kg/day and the fetoembryotoxic NOEL was 60.0 mg/kg/day. The A/D ratio is 1/3.

Classification: CORE MINIMUM

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: alpha-butyl-alpha-(4-chlorophenyl)-1H-1,2,4-triazole)-1-propanenitrile
Description: amber viscous liquid/solid
Batch #(s), Other #(s): Lot # LAP-0298 and TD # 83-260
Purity: 90.4%
Source: Rohm and Haas
Vehicle (if applicable): HiSil 233 carrier suspended in 1% methylcellulose (A4C)

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): New Zealand White Rabbit (female)
Age: 5 months
Weight(s): 2.5-4.2 kg
Source(s): Hazleton Dutchland, Denver, PA

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3. Procedures:

Five groups of 18 female rabbits were used for the study. The following dose levels were tested, based upon a preliminary range-finding study (summarized in a separate report): 0 (water control), 0 (Hi-Sil control), 20.0, 60.0 and 200.0 mg/kg/day a.i. (5 ml/kg b.w.). The test chemical was prepared with the solid adsorbent (carrier) Hi-Sil 233 and with 1.0% methylcellulose (vehicle). The dosing suspensions were prepared daily and samples were retained for analysis. Samples from the first, tenth and last dosing days were actually submitted for analysis. The male rabbits used in the study were of the same strain and from the same source as the females. These animals had been maintained at the facility for breeding purposes. The females were artificially inseminated and the day on which insemination occurred was considered to be day 0 of gestation. Three hours prior to insemination, the does were induced to ovulate with an intravenous injection of chorionic gonadotropin. The test chemical was administered by oral intubation on days 7 through 19 of gestation.

The does were observed for signs of clinical toxicity and mortality at least once daily (twice daily during the dosing period). Body weights were recorded on days 0, 4, 7, 11, 15, 20, 25 and 29 of gestation. On day 29 of gestation the does were sacrificed and the following items were examined and recorded: the number and uterine position of viable and non-viable fetuses, and early and late resorptions. In addition, total implantations were tabulated and recorded and the number of corpora lutea on each ovary was recorded.

Each live fetus was weighed and examined for external malformations and variations. Each was then dissected and internally sexed and examined for visceral malformations and variations. Each brain was examined through a mid-coronal incision and each heart was examined by a modification of the Staples method. All fetuses were skinned, fixed in 95% ethyl alcohol, mascerated in potassium hydoxide and stained with Alizarin red S for subsequent skeletal examination. Variations and malformations were tabulated for the fetuses.

Any data that suggested any treatment-related effects were statistically analyzed. Parametric and nonparametric procedures were used. The various indices (e.g. Fertility, Viability, etc.) were analyzed by the Jonkheere test for dose-related trends followed by pairwise comparisons of treated groups to separate and/or combined controls.

B. RESULTS:

Maternal Observations:

No signs of clinical toxicity were observed in the 20 and 60 mg/kg/day groups when compared to controls. In the 200 mg/kg/day group, increases in the following signs were noted: irregular feces, bloody urine, bloody urogenital or anal area and blood and/or aborted material in the drop pan. The authors state that the incidences of blood or aborted material in the drop pan in the 200 mg/kg group were related to the abortions or total resorptions. The incidences of blood in the pan at 20 and 60 mg/kg were related to abortions

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which occurred in these groups. Two of 18 does in the 200 mg/kg group died due to intubation errors. No other deaths were observed in any of the other groups. The Fertility Index was 0.67 or greater for each group in the study and 0.76 overall. The following number of rabbits/group did not get pregnant: 5/18 (water control), 6/18 (Hi-Sil control), 4/18 (20 mg/kg), 3/18 (60 mg/kg) and 4/18 (200 mg/kg). There were no abortions in the controls and 1 each in the 20 and 60 mg/kg dose groups. These were considered to be incidental abortions and not related to treatment. The conceptuses recovered from these two abortions were early resorptions, suggesting that the fetuses died in utero prior to the abortion. Three does in the 200 mg/kg group aborted their litters. This incidence was considered by the authors to be related to treatment - probably a combined maternal toxicity and an embryofetotoxic effect. All the recovered conceptuses were early resorptions. See Table 7 for details.

No treatment-related difference in body weights was observed in does at the 20 mg/kg level. At 60 mg/kg, it appeared that maternal toxicity occurred with the first exposures to the test chemical, from which the does recovered and resumed a normal growth pattern at a reduced weight. At 200 mg/kg, maternal weight gain during treatment was significantly reduced.

There were no differences observed between any of the treated groups and the controls when the dams were examined at necropsy.

Ovarian, Uterine and Fetal Data

The number of corpora lutea/litter was not significantly different in any of the treated groups when compared to controls, although at 200 mg/kg the number was less (7.56 as opposed to 11.31 and 12.75 for controls). With the exception of the 200 mg/kg group, the numbers were higher than historical control values. The number of corpora lutea/litter for the 200 mg/kg group was within the historical control limits. In addition, the number of implantation sites/litter was within normal limits for all groups when compared to historical control data. There were no dead fetuses in any of the treated groups. In the 20 and 60 mg/kg groups there were no litters with more than 2 resorptions and no litters completely resorbed. The Viability Index for these two groups was not significantly different from combined control values. At a dose level of 200 mg/kg/day, when compared to controls, there was an increased incidence in the number of resorptions/litter, the number of litters with more than 2 resorptions and the number of litters totally resorbed. The Viability Index was significantly reduced when compared to controls. See Table 3 for details.

Litter size was significantly reduced at the 200 mg/kg level. At the other two dose levels, litters sizes were not different from controls. There were no statistically significant differences in fetal weights in any of the treated groups when compared to controls. The fetal sex ratio (# males/# females) was not significantly different in any of the treated groups when compared to combined controls. The higher value (2.38) in the 200 mg/kg group was considered by the authors to be a random occurrence because the number was based upon a small number of litters. The ratios for the controls were 1.48 and 1.03 (water controls and vehicle controls, respectively). See Table 4 for details.

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There were no external variations in any group. The frequencies of the soft tissue variations were not suggestive of an effect at any dose, not was there a dose-response. The frequencies across the groups were within the range of historical controls. There were no significant trends across control and treated groups for any skeletal variations. The percentage of fetuses with some of the more common variations tended to be higher in the 200 mg/kg group than in any of the other groups. This may be due to the fact that resorptions and abortions were so high in this group that the number of fetuses available for examination was approximately one-third of the numbers available in the other groups. The most common skeletal variations observed throughout the groups were: 13th rudimentary ribs (unilateral or bilateral), 13th full ribs (bilateral), 13th full rib with 13th rudimentary rib, and 27 presacral vertebrae. Other variations observed included: bent hyoid arches, accessory nasal bones, 25 presacral vertebrae, sternebra 5 and/or 6 unossified and sternebrae misaligned. No primary external malformations were noted for any of the test groups. Cardiomegally was found in one fetus in one litter at 20 mg/kg. This was considered to be a spontaneous occurrence. No treatment-related skeletal malformations were observed in any of the treated groups. Single occurrences were observed in different dose groups. These included fused skull bones, fused sternebrae, ribs with spherical enlargements, and scoleosis (with or without associated rib anomalies). When all the malformations were considered together, no significant dose-response was observed. See Tables 5 and 6 for details.

The NOEL for maternal toxicity was 20.0 mg/kg/day and the fetoembryotoxic NOEL was 60.0 mg/kg. RH-53,866 was not teratogenic at any dose up to 200.0 mg/kg/day, the highest dose level tested.

C. DISCUSSION:

This study was well conducted. However, only 3 litters were available for examination at the highest dose level. Since the EPA testing guidelines call for at least 12 litters to be available for examination at any dose level, this study is classified as CORE MINIMUM.

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RIN 1067-98

Myclobutanil Tox Review

Page_____ is not included in this copy.

Pages 124 through 128 are not included.

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Reviewed By: Pamela Hurley, Toxicologist Pamela M. Hurley 4/26/94
Section I, Tox. Branch (7509C)
Secondary Reviewer: Roger L. Gardner, Head Roger Gardner 4-26-94
Section I, Tox. Branch (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Developmental Toxicity (83-3) - **Supplemental DER**

SPECIES: Rat

SHAUGHNESSY NO./CASWELL NO.: 128857

ACCESSION NUMBER/MRID NO.: 00141672

TEST MATERIAL: RH-53,866

SYNONYMS: Myclobutanil, Rally, Systhane, Nova

REPORT NUMBER: 83R-024

SPONSOR: Rohm and Haas Company, Spring House, PA

TESTING FACILITY: Rohm and Haas Company, Toxicology Department, Spring House, PA

TITLE OF REPORT: Teratology Study with RH-53,866 in Rats

AUTHOR(S): R.D. Costlow and W. W. Kane

REPORT ISSUED: June 22, 1984

CONCLUSION: In a developmental toxicity study, 25 Sprague-Dawley [Crl:CD-(SD)BR] rats/dose group received either 0, 31.26, 93.77, 312.58 or 468.87 mg/kg/day technical myclobutanil (84.5% a.i.) by oral gavage from gestation day 6 through 15, inclusive. The animals received the test material in a dose volume of 10 ml/kg. The females were mated to proven males on a one-to one basis for up to 5 days. The day on which sperm were noted was designated as gestation day 0.

Maternal toxicity was not observed at either 31.3 or 93.8 mg/kg/day. At 312.6 mg/kg/day, clinical signs of toxicity consisting of rough hair coat and salivation were observed. At 468.87 mg/kg/day, rough hair coat, salivation, alopecia, desquamation and red exudate around the mouth were observed. No other effects were observed. **The maternal toxicity LOEL is 312.6 mg/kg/day and the maternal toxicity NOEL is 93.8 mg/kg/day based on clinical signs of toxicity.**

A significant increase in the incidences of 14th rudimentary ribs and 7th cervical ribs was observed at 312.6 and 468.9 mg/kg/day. No other developmental effects were observed at any dose level tested. **The developmental toxicity LOEL is 312.6 mg/kg/day and**

the developmental toxicity NOEL is 93.8 mg/kg/day based on increased incidences of 14th rudimentary and 7th cervical ribs.

The study is classified as Core Minimum Data (Acceptable) and satisfies the requirement (83-3 a) for a developmental toxicity study in rats.

This is a supplemental DER which includes a re-analysis of the data. The maternal and developmental NOELs and LELs supercede the previous NOELs and LELs.

A. MATERIALS AND METHODS:

Basis For Selection of Dose Levels: The dose levels for the main study were selected on the basis of a range-finding study in which the test material was found to induce toxicity in pregnant rats at dose levels of 464.4 and 700.0 mg/kg (2/8 and 8/8 dams died, respectively, preceded by decreases in body weight gain, lethargy, ataxia, red exudate around the mouth and rough hair coat). Microscopic examinations had revealed reddened intestines and pancreas, reddened and enlarged adrenals, hemorrhagic esophagus and focal erosions in the gastric mucosa. There were no treatment-related maternal effects at dose levels of 215.0 mg/kg/day and below.

B. RESULTS:

Maternal Toxicity:

1. Clinical Observations and Mortality: The following table summarizes selected clinical signs of toxicity observed in the dams during the study.

Effects of RH-53,866 on the Incidence of Selected Pharmacological Observations in Pregnant Rats

Observation	Incidence[a] at Dosage (mg/kg/day) 0	31.3	93.8	312.6	468.9
No. mated ♀	25	25	25	25	25
Normal	22(525)	18(525)	17(525)	13(525)	3(525)
Alopecia	2(20)	7(42)	7(63)	7(67)	15(147)
Rough coat	0(0)	0(0)	1(1)	4(8)	8(27)
Desquamation	0(0)	0(0)	0(0)	1(12)	5(36)
Salivation	0(0)	0(0)	0(0)	3(3)	4(4)
Red exudate from mouth	0(0)	0(0)	0(0)	0(0)	10(22)
Urine stain	1(2)	0(0)	0(0)	1(1)	6(17)

[a] Reported as the number of animals exhibiting the sign. Numbers in parentheses represent the total number of occurrences.

Based on this table and on the clinical signs exhibited by the dams in the range-finding study, it appears that the NOEL for clinical signs is 93.8 mg/kg/day and the LEL is 312.6 mg/kg/day. The LEL is borderline. The incidences of rough coat in 4 dams and the incidences of salivation in 3 dams indicate that these animals may not have been feeling well.

2. Body Weight and Body Weight Gain

The report stated that a statistically significant decrease in maternal body weight was observed on gestation day 10 in the high dose group. For the Data Evaluation Record (DER), the Contractor, Dynamac Corporation conducted a separate statistical analysis and found that there were no statistically significant differences in body weight at any dose level at any time using ANOVA and Dunnett's t-test (single comparisons with controls and tests for trend). In addition, on day 10, the mean body weight at the highest dose level was 97% of the control value and was not considered to be biologically significant. The DER further stated that the authors of the study reported slight decreases in maternal body weight gains (approximately 10.5%) between gestation days 6 and 16 at the highest dose level. However, these decreases in body weight gain were not statistically significant at

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any time. The following table summarizes the body weight gain data.

Body Weight Gain (g) at Selected GD Intervals

Mg/kg/day	0-6	6-16	16-20	0-20
0	31	38	69	138
31.3	22	42	68	132
93.8	25	40	66	131
312.6	24	42	65	131
468.9	27	34	69	130

There were no treatment-related changes in body weight gain data at any dose level. There were no other indications of maternal toxicity in the study.

Developmental Toxicity:

The developmental toxicity data were re-evaluated because in some of the calculations, a litter which was entirely resorbed was included. These numbers have been corrected in the following table summarizing ovarian and uterine data. The re-evaluation also changes the NOEL and LEL for developmental toxicity.

The Effect of RH-53,866 on Reproductive Parameters in Rats

Parameters	Dosage (mg/kg/day) 0	31.3	93.8	312.6	468.9
# Females mated	25	25	25	25	25
# Females pregnant	22	24	21	23	23
Pregnancy rate (%)	88	96	84	92	92
# Corpora lutea/litter	17.9	15.2	16.6	16.4	16.8
# Implantations/litter	16.1	14.3*	15.2	15.0	15.7
Implantation efficiency	0.91	0.94	0.93	0.91	0.94
# Live fetuses/litter	15.3 ±0.6[c]	13.5* ±0.4	13.3* ±0.7	13.2* ±0.7	13.7* ±0.4[d]
Viability index[a]	0.95	0.95	0.88*	0.88*	0.87*[d]
Mean No. resorptions	0.82 ±0.20[c]	0.79 ±0.12	1.86 ±0.57	1.78* ±0.34	2.05*[d] ±0.35
Mean early resorptions	0.82	0.71	1.76	1.35	1.50
Mean late resorptions	0.00	0.08	0.10	0.43	0.55
Mean fetal body wt. (g)	3.23	3.30	3.25	3.39	3.26
Fetal sex ratio[b]	0.99	1.14	1.07	1.43	0.94

a No. live fetuses/No. implantation sites
b No. male fetuses/No. female fetuses
c Standard error of the mean with N equally number pregnant.
d Calculation does not include one litter in which all fetuses were resorbed.
* Significantly different from controls at $p<0.05$

As can be seen from the table, there appear to be effects on viability index and possibly mean number of resorptions (increased but not statistically significant at 93.8 mg/kg/day) at dose levels down to 93.8 mg/kg/day. These effects are probably not biologically significant because the litter sizes for all the treated groups are all equivalent (there is no dose-response) and they are within the historical control range (see below). The litter size for the control group is higher than the historical control range (again, see below). In addition, when the individual animal data are examined for the treated groups, for those litters in which there were a greater number of resorptions, there were also a larger number of corpora lutua and/or implantations in the other litters where there were fewer resorptions (see individual animal data below). This adjustment did not occur in the control group, although the mean number of resorptions per dam were at the top of the

historical control range. At the lowest dose level (31.3 mg/kg/day), there appears to be a decrease in the number of live fetuses/litter. This effect is also not biologically significant because the mean number of corpora lutea and implantations for this group were lower to begin with. In addition, the implantation efficiency was higher than the controls and the mean number of resorptions and the viability index for this group were either equivalent to (viability index) or less than the control group (resorptions).

These data are supported by the historical control information which was provided from 6 studies as of March, 1984. These data were collected from 90 gravid females. The individual values for the number of dams in each study were not provided. Selected data are as follows:

No. of dams with resorptions only:	0
No. of dams with live fetuses:	90
No. of live fetuses/dam:	13.46 (12.10 - 14.6)
No. of implantations/dam:	13.82 (12.91 - 14.92)
No. of corpora lutea/dam:	15.45 (14.82 - 17.4)
No. of late resorptions/dam:	0.02 (0.00 - 0.05)
No. of early resorptions/dam:	0.56 (0.32 - 0.82)

As can be seen from the data, the number of live fetuses/dam, implantations/dam and corpora lutea/dam for the controls in the present study were above and beyond the range of the historical controls. For all of the treated groups, the number of live fetuses/litter were within the historical control range. It is noted here that in the report, for the high dose group, 14 resorptions from a litter that was totally resorbed were included in the calculation for resorptions/litter. These have been left out of the calculation here and are treated as a separate case. A tabulation of the individual animal data provides the following information on resorptions:

Resorption Data for Rat Developmental Toxicity Study

Dose (mg/kg)	0	31.3	93.8	312.6	468.9
Total # litters	22	24	21	23	22[b]
# Dead fetuses	0	0	1	0	0
No. litters totally resorbed	0	0	0	0	1
Total resorptions/dam	0.82	0.79	1.86	1.78	2.05[b]
No. litters with no resorptions	10	7	5	5	4
No. litters with 1 resorption	8	15	9	6	6
No. litters with 2 resorptions	2	2	2	7	5
No. litters with 3 resorptions	2	0	2	2	2
No. litters with 4 resorptions or more	0	0	3	3	5
Total resorptions	18	19	39[a]	41	45[b]

[a] = 1 litter had 10 early and 2 late resorptions out of a litter of 15.
[b] = Not including 1 litter in which all 14 fetuses were resorbed.

Ovarian and Uterine Data - Individual Litters
Group 1 - 0.0 mg/kg/day Vehicle Control

Maternal Number	Codes	Total Corpora Lutea	Total Implantations	Total Viable	Resorptions Early	Resorptions Late	Resorptions Total
83-04022		19	19	18	1	0	1
83-04023		18	18	17	1	0	1
83-04024		15	15	15	0	0	0
83-04025		17	16	13	3	0	3
83-04026		19	19	17	2	0	2
83-04027	Non-gravid	.	0	0	0	0	0
83-04028		19	19	16	3	0	3
83-04029	Non-gravid	.	0	0	0	0	0
83-04030		19	5	5	0	0	0
83-04031		15	13	12	1	0	1
83-04032		19	18	17	1	0	1
83-04033		16	15	13	2	0	2
83-04034		17	17	17	0	0	0
83-04035		19	17	16	1	0	1
83-04152		19	18	18	0	0	0
83-04037		18	18	17	1	0	1
83-04038		19	16	16	0	0	0
83-04039		19	18	18	0	0	0
83-04040		13	13	13	0	0	0
83-04041		16	15	15	0	0	0
83-04042	Non-gravid	.	0	0	0	0	0
83-04043		17	17	16	1	0	1
83-04044		18	18	18	0	0	0
83-04045		25	15	15	0	0	0
83-04047		17	16	15	1	0	1
Mean		17.9	16.1	15.3	0.82	0.00	0.82
S.E.M.		0.49	0.65	0.62	0.20	0.00	0.20
N		22	22	22	22	22	22

. = Missing due to non-gravid
All dams pregnant except as noted. Dams which were non-gravid were not included in calculating means.

Ovarian and Uterine Data - Individual Litters
Group 2 - 31.3 mg/kg/day

Maternal Number	Codes	Total Corpora Lutea	Total Implantations	Total Viable	Resorptions Early	Resorptions Late	Resorptions Total
83-04048	Non-gravid	.	0	0	0	0	0
83-04049		16	15	15	0	0	0
83-04050		18	17	16	1	0	1
83-04051		14	13	11	2	0	2
83-04052		13	13	12	1	0	1
83-04053		19	17	17	0	0	0
83-04054		16	15	14	1	0	1
83-04055		15	15	14	1	0	1
83-04056		16	16	15	1	0	1
83-04057		17	15	14	1	0	1
83-04058		15	13	13	0	0	0
83-04059		16	14	14	0	0	0
83-04060		13	13	12	1	0	1
83-04061		13	13	12	1	0	1
83-04062		16	16	15	1	0	1
83-04063		12	10	10	0	0	0
83-04064		15	15	15	0	0	0
83-04065		13	13	12	1	0	1
83-04066		17	17	16	0	1	1
83-04067		14	13	12	1	0	1
83-04068		16	16	15	1	0	1
83-04069		14	9	9	0	0	0
83-04070		17	17	15	1	1	2
83-04071		14	14	13	1	0	1
83-04072		15	15	14	1	0	1
Mean		15.2	14.3	13.5	0.71	0.08	0.79
S.E.M.		0.36	0.42	0.40	0.11	0.06	0.12
N		24	24	24	24	24	24

. = Missing due to non-gravid
All dams pregnant except as noted. Dams which were non-gravid were not included in calculating means.

Ovarian and Uterine Data - Individual Litters
Group 3 - 93.8 mg/kg/day

Maternal Number	Codes	Total Corpora Lutea	Total Implantations	Total Viable	Resorptions Early	Resorptions Late	Resorptions Total
83-04073		20	18	14	4	0	4
83-04074		15	15	3	10	2	12
83-04075		15	14	13	1	0	1
83-04076		23	15	14	1	0	1
83-04077		16	15	15	0	0	0
83-04153		19	18	16	2	0	2
83-04079	Non-gravid	.	0	0	0	0	0
83-04080		15	14	11	3	0	3
83-04081		12	12	12	0	0	0
83-04082		15	15	14	1	0	1
83-04083		16	15*	14	0	0	0
83-04084		16	14	13	1	0	1
83-04085		15	15	15	0	0	0
83-04086		16	16	12	4	0	4
83-04087		15	15	14	1	0	1
83-04088		19	16	14	2	0	2
83-04089	Non-gravid	.	0	0	0	0	0
83-04090		22	18	17	1	0	1
83-04091		16	16	16	0	0	0
83-04092		11	10	9	1	0	1
83-04093		18	17	16	1	0	1
83-04094	Non-gravid	.	0	0	0	0	0
83-04095		18	17	14	3	0	3
83-04096	Non-gravid	.	0	0	0	0	0
83-04098		17	15	14	1	0	1
Mean		16.6	15.2	13.3	1.76	0.10	1.86
S.E.M.		0.63	0.42	0.65	0.49	0.10	0.57
N		21	21	21	21	21	21

. = Missing due to non-gravid. * Includes one dead fetus.
All dams pregnant except as noted. Dams which were non-gravid were not included in calculating means.

Ovarian and Uterine Data - Individual Litters
Group 4 - 312.6 mg/kg/day

Maternal Number	Codes	Total Corpora Lutea	Total Implantations	Total Viable	Resorptions Early	Resorptions Late	Resorptions Total
83-04155		16	15	15	0	0	0
83-04101		17	17	10	6	1	7
83-04102		21	18	16	2	0	2
83-04103		15	15	13	2	0	2
83-04104		19	19	15	4	0	4
83-04105		13	8	7	0	1	1
83-04106		13	13	12	1	0	1
83-04107		15	15	15	0	0	0
83-04099		20	19	18	1	0	1
83-04109		16	15	12	1	2	3
83-04110		18	15	13	2	0	2
83-04111	Non-gravid	.	0	0	0	0	0
83-04112		20	14	12	2	0	2
83-04113		18	17	14	2	1	3
83-04117		17	17	15	1	1	2
83-04118		18	15	13	0	2	2
83-04114		15	15	14	0	1	1
83-04115		17	17	17	0	0	0
83-04116		16	16	16	0	0	0
83-04119		14	14	14	0	0	0
83-04120		18	18	16	1	1	2
83-04121		12	12	8	4	0	4
83-04122		16	15	14	1	0	1
83-04123		14	5	4	1	0	1
83-04124	Non-gravid	.	0	0	0	0	0
Mean		16.4	15	13.2	1.35	0.43	1.78
S.E.M.		0.50	0.68	0.69	0.32	0.14	0.34
N		23	23	23	23	23	23

. = Missing due to non-gravid
All dams pregnant except as noted. Dams which were non-gravid were not included in calculating means.

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Ovarian and Uterine Data - Individual Litters
Group 5 - 468.9 mg/kg/day

Maternal Number	Codes	Total Corpora Lutea	Total Implantations	Total Viable	Resorptions Early	Resorptions Late	Resorptions Total
83-04125		15	14	14	0	0	0
83-04126		22	17	14	2	1	3
83-04127		17	16	15	1	0	1
83-04128		17	15	13	2	0	2
83-04130		18	17	15	1	1	2
83-04131		17	17	13	4	0	4
83-04132		17	17	16	1	0	1
83-04133	Non-gravid	.	0	0	0	0	0
83-04134		20	16	11	5	0	5
83-04135		14	14	12	2	0	2
83-04136		15	15	15	0	0	0
83-04137		14	14	13	0	1	1
83-04139		17	17	15	2	0	2
83-04140		17	17	17	0	0	0
83-04141		16	16	14	1	1	2
83-04142		17	16	13	2	1	3
83-04143		18	15	14	0	1	1
83-04144		17	17	13	2	2	4
83-04145		14	14	13	1	0	1
83-04147		17	16	11	5	0	5
83-04149		18	15	15	0	0	0
83-04150		17	17	16	1	0	1
83-04151		A	14	0	14	0	14[a]
83-04156	Non-gravid	.	0	0	0	0	0
83-04158		15	14	9	1	4	5
Mean		16.8	15.7	13.1	2.04	0.52	2.57
S.E.M.		0.41	0.26	0.71	0.62	0.20	0.62
N		22	23	23	23	23	23

. = Missing due to non-gravid
All dams pregnant except as noted. Dams which were non-gravid were not included in calculating means.
[a]All implants were resorbed. The numbers in this table include the resorbed litter.
A = Inadvertently not counted at C-section.

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C. DISCUSSION:

The maternal toxicity data indicate that there may be an effect of 312.6 mg/kg/day, based on clinical signs of toxicity. The NOEL for maternal toxicity is 93.8 mg/kg/day and the LEL is 312.6 mg/kg/day. This LEL is a borderline LEL because the effects were not prominent and were not supported by other effects such as decreases in body weight and body weight gain.

The developmental toxicity data are deceiving and appear to indicate an effect. However, when the data are examined, the litter sizes are within the historical control range for this strain of rat. The mean number of corpora lutea and implantation sites and litter size for the control group were higher than the historical control range, thus giving a false impression of an effect. However, it is not known why the control group did not adjust its litter sizes as the treated groups did. Therefore, the NOEL for developmental toxicity is based on the increased incidences of 14th rudimentary and 7th cervical ribs at 312.6 mg/kg/day and above: 93.8 mg/kg/day (see original DER and addendum to DER).

EPA: 68-02-4225
DYNAMAC No. 33-01,2
December 31, 1985

DATA EVALUATION RECORD

RH-53,866

Teratogenicity Study in Rats

STUDY IDENTIFICATION: Costlow, R. D. and Kane, W. W. Teratology study with RH-53,866 in rats. (Unpublished study No. 83R-024 prepared and submitted by Rohm and Haas Company, Spring House, PA; dated June 22, 1984.) Accession No. 072901.

APPROVED BY:

I. Cecil Felkner, Ph.D.
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Dynamac Corporation

Signature: I. Cecil Felkner

Date: 12-31-85

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1. CHEMICAL: RH-53,866; alpha-butyl-alpha-4-chlorophenyl-1H-1,2,4-triazole-1-propanenitrile.

2. TEST MATERIAL: RH-53,866 (technical), lot No. LSPL 83-0017E, TD No. 83-087, was described as a viscous brown liquid consisting of 84.7% active ingredient.

3. STUDY/ACTION TYPE: Teratogenicity study in rats.

4. STUDY IDENTIFICATION: Costlow, R. D. and Kane, W. W. Teratology study with RH-53,866 in rats. (Unpublished study No. 83R-024 prepared and submitted by Rohm and Haas Company, Spring House, PA; dated June 22, 1984.) Accession No. 072901.

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Addendum to Teratology Study in Rats Exposed to RH-53,866

The secondary reviewer, Dr. Jane E. Harris, disagrees with this interpretation that the increased incidences of 7th cervical ribs at the two highest doses are suggestive of a teratogenic potential of RH-53,866. Rather, the presence of such developmental variations as increased incidences of 7th cervical ribs and 14th rudimentary ribs in conjunction with the observed embryotoxicity, namely, an increased number of resorptions and decreased viability index suggest a fetotoxic effect. The absence of an increased incidence of soft tissue or skeletal malformations in rats exposed to RH-53,866 at doses up to 468.9 mg/kg support the conclusion that the increased incidences of 7th cervical and 14th rudimentary ribs at the two highest doses are developmental variations associated with fetotoxic effects.

7. CONCLUSIONS:

A. The LOEL and NOEL for maternal toxicity in rats given oral doses of RH-53,866 from days 6 through 15 of gestation are 468.9 and 312.6 mg/kg/day respectively, based on slight decreases in body ~~weight~~ gain from GD 6-16 and clinical signs such as rough hair coat, salivation, alopecia, desquamation and red exudate around the mouth observed at 468.9 mg/kg/day after initiation of dosing.

The LOEL and NOEL for embryo/fetotoxicity are 93.8 and 31.3 mg/kg/day, respectively, based on slight increases in the mean number of resorptions noted at 93.8 and 312.6 mg/kg/day, marked increases in resorptions at 463.9 mg/kg/day, significant decreases in viability indices at 93.8 mg/kg/day and above, and significantly increased incidences of 14 rudimentary ribs and 7th cervical ribs at 312.6 and 468.9 mg/kg/day.

Since seventh cervical ribs are rare in rats, and since this anomaly is associated with clinical complications (nerve and blood vessel obstruction in the neck region), we assess that the dose-related increase of this finding suggests a teratogenic potential of RH-53,866.[2]

B. This study is classified as Core Minimum.

Item 8--see footnote 1.

9. BACKGROUND:

A range-finding teratogenicity study was conducted in rats to determine appropriate doses of RH-53,866 for a subsequent teratogenicity study. Daily oral dosages of 0, 31.6, 68.1, 100.0, 215.0, 464.4, or 700.0 mg/kg of test material in corn oil were administered to seven groups of eight mated Sprague-Dawley rats (Charles River) from gestational days (GD) 6 through 15.

All eight females in the 700-mg/kg dose group died before GD 20 (study termination), and two of eight females died in the 464.0-mg/kg dose group. Death was preceded by decreases in body weight, lethargy, ataxia, red exudate around the mouth, and rough hair coat. Maternal body weights were significantly decreased ($p < 0.05$) from GD 10 through study termination in the 464.4-mg/kg dose group when compared to controls. Findings at necropsy for the animals that died in the

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[1] Only items appropriate to this DER have been included.

2. See Addendum

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in the 700-mg/kg dose group included reddened intestines, reddened and enlarged adrenals, reddened pancreas, hemorrhagic esophagus, and focal erosions in gastric mucosa, indicating that RH-53,866 at this dose level caused gastrointestinal irritation and stress. No compound-related maternal effects were noted at dosages below 464.4 mg/kg/day.

Pregnancy rates, number of corpora lutea and implantations, and implantation efficiency were comparable among control and test groups. The number of viable fetuses at 215.0 and 464.4 mg/kg and the viability index at 68.1 and 464.4 mg/kg of RH-53,866 were lower than controls. The number of resorptions was increased in all test groups compared to controls.

From results of this range-finding study, the authors concluded that the RH-53,866 was toxic to pregnant rats at 464.4 and 700.0 mg/kg.

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. Test Material: Daily aliquots of technical RH-53,866, lot No. LSPL 83-0017E (84.5% active ingredient), were heated to 55°C, and 0.370, 1.1098, 3.6991, and 5.5487 g (adjusted for purity) of the test material were measured into volumetric flasks. The volumes were then adjusted to 100 mL by adding preheated (55°C) corn oil to obtain concentrations of 0, 31.26, 93.77, 312.58, and 468.87 mg/kg when administered in a dose volume of 10 mL/kg. The dose formulations were stirred continuously during dosing using a magnetic stir plate and were administered orally on GD 6 through 15; volumes were adjusted on weighing days for changes in body weight.

   Samples of the dose formulations were collected daily and analysis was conducted on the first, eighth, and last day of dosing. The methodology for analysis was not specified.

2. Animals and Test System: Virgin female Sprague-Dawley [Crl:CD-(SD)BR] rats approximately 63 days of age were received from Charles River Breeding Laboratories, Stone Ridge, NY, assigned temporary animal numbers, and acclimated for 20 days. During the acclimation period, animals within a body weight range of 2 standard deviations above or below the mean of the entire group of acclimating females were selected for the study. The animals were then randomized and assigned unique permanent numbers. At the end of the acclimation period, the females were placed in five groups of 25 animals each from lowest to highest consecutive number and mated to proven males of the same strain and source on a one-to-one

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basis for up to 5 days. Eight "extra" females were mated and used as replacements for females on study that did not mate within 5 days. Vaginal smears were performed daily to confirm matings; the day on which sperm was noted was designated as GD 0.

The animals were observed daily for signs of mortality, toxicity, and general health, and body weights were measured on GD 0, 6, 10, 13, 16, 18, and 20.

Dams were sacrificed on GD 20 using carbon dioxide, and fetuses were delivered by Cesarean section. The uteri were examined for number and position of live and dead fetuses and early and late resorptions. The number of corpora lutea was recorded. Dams were then examined for visceral abnormalities; maternal tissues having gross lesions were fixed in 10% neutral-buffered formalin for possible histological examination. Fetuses were individually tagged according to protocol number, dam's identification number, and uterine position. Each live fetus was then weighed, sexed, and examined for external abnormalities. The authors did not specify the method of fetal sacrifice. Two-thirds of the fetuses from each litter were eviscerated, fixed in 95% ethyl alcohol, macerated in 2% aqueous potassium hydroxide, stained with Alizarin red S, and examined for skeletal abnormalities. The remaining one-third of the fetuses were fixed in Bouin's solution, sectioned, and examined for visceral abnormalities.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

A. Test Material Analysis: The authors reported an error in the preparation of the dose formulations. The value for percent active ingredient used in the calculations was 81.1. However, the test material used in this study, lot No. LSPL 83-0017E, consisted of 84.5% active ingredient. The doses administered were therefore 4.19% higher than originally intended. The data were changed to reflect the adjustments; the actual doses were 31.3, 93.8, 312.6, and 468.9 mg/kg of RH-53,866.

Results of the chemical analysis indicated that the actual doses were between 88.3 and 102.7% of the target concentrations.

B. Maternal Effects: No mortalities occurred during the study. However, the following pharmacological observations were noted: rough hair coat, desquamation, salivation, alopecia, and urine stain. A summary of clinical observations is presented in Table 1. In addition, red exudate from the vagina and scant feces were exhibited by one and three animals, respectively, from the 468.9-mg/kg dose group. The authors considered the increased

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TABLE 1. Effects of RH-53,866 on the Incidence of Selected Pharmacological Observations in Pregnant Rats

Observation	Incidence[a] at Dosage (mg/kg/day)				
	0	31.3	93.8	312.6	468.9
Number of mated females	25	25	25	25	25
Normal	22(525)	18(525)	17(525)	13(525)	3(525)
Alopecia	2 (20)	7 (42)	7 (63)	7 (67)	15(147)
Rough coat	0 (0)	0 (0)	1 (1)	4 (8)	8 (27)
Desquamation	0 (0)	0 (0)	0 (0)	1 (12)	5 (36)
Salivation	0 (0)	0 (0)	0 (0)	3 (3)	4 (4)
Red exudate from mouth	0 (0)	0 (0)	0 (0)	0 (0)	10 (22)
Urine stain	1 (2)	0 (0)	0 (0)	1 (1)	6 (17)

[a]Reported as the number of animals exhibiting the sign. Numbers in parentheses represent the total number of occurrences.

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incidences of rough hair coat, desquamation, salivation, urine stain, and the presence of red exudate from the mouth to be compound-related effects. The authors discounted the alopecia, stating that hair loss frequently occurs in rats during gestation.

The authors reported slight decreases in maternal body weight gains (approximately 11%) between GD 6 through 16 of dosing at the 468.9-mg/kg level, but the only significant decrease occurred on GD 10 (Table 2). They further stated that a significant decrease in mean body weight occurred in the 31.3-mg/kg group on GD 13. However, statistical analysis of body weights by these reviewers revealed no significant differences in any dose group when compared to controls. Body weight gains were comparable between controls and the 93.8- and 312.6-mg/kg dose groups. No compound-related changes were observed at necropsy.

C. Embryonic/Fetal Effects: Pregnancy rates were comparable between control and test groups (Table 3). The authors stated that the numbers of corpora lutea and implantations for the test groups were within the range for historical controls, but values for the concurrent control group were slightly higher than those for historical controls; however, no statistical analyses were conducted on these parameters. Statistical analysis of implantation efficiencies revealed no differences between control and test groups. There were significant decreases ($p < 0.05$) in the number of viable fetuses per litter in all dose groups and the viability indices for the 93.8-, 312.6-, and 468.9-mg/kg dose groups when compared to controls (Table 3). No differences in fetal body weights or sex ratios occurred between control and test groups.

The effect of RH-53,866 on the incidences of variations and malformations is presented in Tables 4 and 5. Significant increases ($p < 0.05$) in the incidences of 7th cervical and 14th rudimentary ribs occurred in the 312.6- and 468.9-mg/kg dose groups when compared to controls. In addition, a significant dose-related trend of reduced ossification was reported, although no individual test group was significantly different from control values. No malformations were observed in the control or 312.6-mg/kg groups. Malformations reported in the 31.3-mg/kg group were microphthalmia in one fetus and atlo-occipital anomaly in a second fetus from a different litter. Several malformations occurred in the 93.8-mg/kg dose group; agnathia, anophthalmia, open eyelids, and misplaced pinna occurred in one fetus from one litter and an interventricular septal defect, retroesophageal aortic arch, and single atrium with a single atrioventricular valve occurred in one fetus from another litter. Craniorachischisis, a vertebral centra anomaly, and two incidences of hydrocephaly occurred in the 468.9-mg/kg dose group. Four fetuses from separate litters were affected, and the authors considered these findings random occurrences that were not compound related. While the number of malformed fetuses at 468.9 mg/kg was significantly higher ($p < 0.05$) than controls and a "marginally" significant dose-related trend was reported, the authors did not consider these findings toxicologically significant. No other compoundrelated changes occurred at any dose level.

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TABLE 2. The Effect of RH-53,866 on the Mean Maternal Body Weights in Rats

Dosage (mg/kg/day)	Body Weight (g) at GD 0	6	10	13	16	20
0	261 ±15.9	292 ±16.9	296 ±17.4	310 ±17.8	330 ±23.5	399 ±31.0
31.3	259 ±14.2	281 ±18.1	288 ±16.7	307* ±21.1	323 ±20.6	391 ±24.0
93.8	255 ±14.7	280 ±15.6	286 ±17.9	299 ±17.4	320 ±20.6	386 ±23.8
312.6	259 ±15.8	283 ±16.8	290 ±20.6	304 ±21.1	325 ±22.1	390 ±26.9
468.9	262 ±13.9	289 ±15.3	288* ±16.8	305 ±15.8	323 ±18.7	392 ±28.8

Dosage (mg/kg/day)	Body Weight Gain (g) at GD Interval 0-6	6-16	16-20	0-20
0	31	38	69	138
31.3	22	42	68	132
93.8	25	40	66	131
312.6	24	42	65	131
468.9	27	34	69	130

*Significantly different from controls at $p < 0.05$. Statistical analysis by these reviewers using ANOVA and Dunnett's t-test revealed no significant differences for any dosage group when compared to controls and no dose-related trend towards decreased body weight gain.

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TABLE 3. The Effect of RH-53,866 on Reproductive Parameters in Rats

Parameters	Dosage (mg/kg/day)				
	0	31.3	93.8	312.6	468.9
No. females mated	25	25	25	25	25
No. females pregnant	22	24	21	23	23
Pregnancy rate (%)	88	96	84	92	92
Mean No. corpora lutea/litter	17.9	15.2	16.6	16.4	16.8
Mean No. implantations/litter	16.1	14.3	15.2	15.0	15.7
Implantation efficiency	0.91	0.94	0.93	0.91	0.94
Mean No. live fetuses/litter	15.3	13.5*	13.3*	13.2*	13.1*
Viability index[a]	0.95	0.95	0.88*	0.88*	0.83*
Mean No. resorptions	0.82	0.79	1.86	1.78	2.57
Mean fetal body wt. (g)	3.23	3.30	3.25	3.39	3.26
Fetal sex ratio[b]	0.99	1.14	1.07	1.43	0.94

[a] $\frac{\text{No. live fetuses}}{\text{No. implantation sites}}$

[b] $\frac{\text{No. male fetuses}}{\text{No. female fetuses}}$

*Significantly different from controls at $p < 0.05$.

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TABLE 4. The Effect of RH-53,866 on Skeletal Findings in Rat Fetuses

	Number (% Incidence) at Dosage (mg/kg/day)				
	0	31.3	93.8	312.6	468.9
No. fetuses examined	223	213	185	200	201
No. litters examined	22	24	21	23	22
7th cervical rib					
Fetal	3(1.3)	9(0)	3(1.6)	17 (8.5)	45(22.4)
Litter	2(9.1)	0(0)	3(14.3)	10(43.5)*	14(63.6)*
14th rudimentary rib					
Fetal	1(0.4)	4(1.9)	1(0.5)	17 (8.5)	72(35.8)
Litter	1(4.5)	3(12.5)	1(4.8)	8(34.8)*	18(81.8)*
Reduced ossification of vertebrae					
Fetal	3(1.3)	1(0.5)	6 (3.2)	5 (2.5)	6 (3.0)
Litter	3(9.1)	1(4.2)	5(23.8)	4(17.4)	5(22.7)
Sternebrae not ossified[a]					
Fetal	8 (3.6)	2(0.9)	3 (1.6)	3(1.5)	8 (4.0)
Litter	5(22.7)	2(8.3)	3(14.3)	2(8.7)	7(31.8)
Sternum not ossified					
Fetal	1(0.4)	0(0)	0(0)	2(1.0)	0(0)
Litter	1(4.5)	0(0)	0(0)	2(8.7)	0(0)

*Significantly different from controls at $p < 0.05$.

[a]Sternebrae other than 5/6.

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TABLE 5. The Effect of RH-53,866 on the Incidences of Malformations in Rat Fetuses

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	Number (% Incidence) at Dosage (mg/kg/day)				
	0	31.3	93.8	312.6	468.9
No. fetuses examined	223	213	185	200	201
No. litters examined	22	24	21	23	22
External Malformations:					
Craniorachischisis					
Fetal	0(0)	0(0)	0(0)	0(0)	1(0.5)
Litter	0(0)	0(0)	0(0)	0(0)	1(4.5)[c]
Agnathia					
Fetal	0(0)	0(0)	1(0.5)[a]	0(0)	0(0)
Litter	0(0)	0(0)	1(4.8)	0(0)	0(0)
Misplaced pinna					
Fetal	0(0)	0(0)	1(0.5)[a]	0(0)	0(0)
Litter	0(0)	0(0)	1(4.8)	0(0)	0(0)
Skeletal Malformations:					
Atlo-occipital anomaly					
Fetal	0(0)	1(0.5)	0(0)	0(0)	0(0)
Litter	0(0)	1(4.2)	0(0)	0(0)	0(0)
Vertebral centra anomaly					
Fetal	0(0)	0(0)	0(0)	0(0)	1(0.5)[d]
Litter	0(0)	0(0)	0(0)	0(0)	1(4.5)
Soft Tissue Malformations:					
Hydrocephaly					
Fetal	0(0)	0(0)	0(0)	0(0)	2(2.0)
Litter	0(0)	0(0)	0(0)	0(0)	2(9.1)
Open eyelids					
Fetal	0(0)	0(0)	1(0.5)[a]	0(0)	0(0)
Litter	0(0)	0(0)	1(4.8)[a]	0(0)	0(0)
Interventricular septal defect					
Fetal	0(0)	0(0)	1(0.5)[a]	0(0)	0(0)
Litter	0(0)	0(0)	1(4.8)	0(0)	0(0)
Atrium with single atrioventricular valve					
Fetal	0(0)	0(0)	1(0.5)[b]	0(0)	0(0)
Litter	0(0)	0(0)	1(4.8)	0(0)	0(0)
Retroesophageal aortic arch					
Fetal	0(0)	0(0)	1(0.5)[b]	0(0)	0(0)
Litter	0(0)	0(0)	1(4.8)	0(0)	0(0)

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[a,b,c,d]The same superscript indicates the same fetus.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that the LOEL and NOEL for maternal toxicity in rats were 312.6 and 93.8 mg/kg of RH-53,866, respectively, based on clinical signs such as rough hair coat, urine stain, and salivation that developed at 312.6 and 468.9 mg/kg after initiation of dosing.

The authors stated that significant decreases in the numbers of live fetuses between control and test groups were not toxicologically significant because the mean litter size for the controls was greater than the range seen in historical control data whereas the values for the test groups were within the historical range. However, the authors considered the statistically significant differences in the viability index at the 93.8-, 312.6-, and 468.9-mg/kg doses to be compound-related occurrences. The authors concluded that the LOEL and NOEL for embryotoxicity in rats were 93.8 and 31.3 mg/kg of RH-53,866, respectively, based on significant decreases in the viability index and increases (not statistically analyzed) in the number of resorptions at 93.8, 312.6, and 468.9 mg/kg of RH-53,866. The LOEL and NOEL for fetotoxicity in rats were assessed to be 312.6 and 93.8 mg/kg, respectively, based on increases in developmental variations such as the 14th rudimentary and 7th cervical ribs at the 312.6- and 468.9-mg/kg dose levels and a significant dose-related trend of reduced ossification. According to the authors, teratogenic effects of RH-53,866 were not evident in this study. The significant increase in malformations reported in the 468.9-mg/kg dose group was not considered a teratogenic response because only four fetuses from separate litters were affected and the types of malformations that occurred were not related.

B. A signed quality assurance statement was present but not dated.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. 1. Maternal Effects: After initiation of dosing, clinical signs such as rough hair coat, salivation, alopecia, desquamation, and urine stains on a urogenital area were observed in the 312.6- and 468.9-mg/kg dose groups. Incidences of red exudate around the mouth and vagina were also observed in the high-dose group after initiation of dose administration suggesting minimal toxicity. In addition, small decrease in body weight gain was noted between 6 through 16 at the 468.9 mg/kg when compared to controls. Therefore, it appears that a more appropriate LOEL for maternal toxicity is 468.9 mg/kg with a NOEL of 312.6 mg/kg.

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2. Embryonic/Fetal Effects: Pregnancy rates, number of corpora lutea, implantations, and implantation efficiency were comparable among control and test groups. The number of resorptions per litter was increased at the 93.8-, 312.6-, and 468.9-mg/kg doses; most resorptions occurred early in gestation, indicating an embryolethal effect. The number of viable fetuses per litter and the viability indices were significantly reduced ($p < 0.05$) at the 31.3-, 93.8-, 312.6-, and 468.9-mg/kg doses and the 93.8-, 312.6-, and 468.9-mg/kg doses, respectively, when compared to controls. We consider the increased incidence of resorptions and decreased litter viability evidence of embryonic/fetal lethality. Fetal body weights and sex ratios were comparable among control and test groups. The incidences of 7th cervical and 14th rudimentary ribs were significantly increased ($p < 0.05$) at 312.6 and 468.9 mg/kg. Although severe malformations such as agnathia, craniorachischisis, and hydrocephaly appeared only in the dose groups and there appeared to be a significant increase in the incidence in malformations in the 468.9-mg/kg dose group when compared to controls, these occurred in very low incidences and not in a dose-related pattern. Therefore, we were unable to make a definitive assessment of the biological significance of these findings.

B. The following items are differences between the reviewers and study authors' interpretation and conclusions:

1. The study authors stated that since alopecia was generally observed in rats during gestation, the increased incidences of hair loss in the test groups were not toxicologically significant. However, the occurrence of alopecia at the 312.6- and 468.9-mg/kg doses was accompanied by desquamation. That is, desquamation occurred only in areas where hair loss was evident; this is not a normal occurrence in rats during gestation. Also, the incidence of alopecia was 2, 7, 7, 7, and 15 for the 0-, 363-, 93.8-, 312.6-, and 468.9-mg/kg groups, respectively, suggesting a compound-related increase; therefore, we assess that the alopecia observed is compound related.

2. We disagree with the study authors' conclusion that decreases in the number of viable fetuses per litter were artifactual and not toxicologically significant. Because the number of corpora lutea, implantations, and implantation efficiency were comparable between the control and test groups, we conclude that the smaller litter sizes in the test groups were due to compound administration. Furthermore, we do not think that comparison of data from the test groups to historical control data instead of to concurrent control data is conclusive.

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3. Although the presence of 7th cervical ribs was considered by the authors to be indicative of fetotoxicity, we consider this finding as a permanent anatomical abnormality that is often associated with adverse physiological complications (nerve and blood vessel obstruction); in addition, this anomaly is rare in rats. Therefore, we assess that the dose-related increase of 7th cervical ribs suggests a teratogenic potential of the test material.*

C. The following deficiencies in the conduct and reporting of this study were noted:

1. The method of fetal sacrifice (if any) was not reported. Therefore, we were unable to determine if the method used was appropriate for a teratology study.

2. Individual fetal and litter body weights were not reported. Therefore, we were unable to independently verify data in the summary tables.

3. The method for confirming pregnancy status (when no visible implantation sites were observed) was not reported. We were, therefore, unable to assess if the method was appropriate for a teratology study.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Study Protocol, CBI pp. 94-105.

* See Addendum

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Reviewed by: Pamela M. Hurley, Toxicologist Pamela M. Hurley 4/12/94
Section I, Toxicology Branch I (7509C)
Secondary Reviewer: Roger L. Gardner, Section Head
Section I, Toxicology Branch I (7509C) Roger Gardner 4/13/94

DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction - Rat (**Supplemental DER**)

TOX. CHEM. NO./SHAUGHNESSY NO.: 723K / 128857

MRID NUMBER: 00143766 and 00149581

TEST MATERIAL: RH-53,866

SYNONYMS: Myclobutanil, Rally, Systhane, Nova

REPORT NO.: 84R-117

SPONSOR: Rohm and Haas Company, Spring House, PA

TESTING FACILITY: Rohm and Haas Company, Toxicology Department, Spring House, PA

TITLE OF REPORT: RH-53,866: Two Generation Reproduction Study in Rats

AUTHOR(S): R. D. Costlow and J. C. Harris

DATE REPORT ISSUED: August 21, 1985

CONCLUSIONS: RH-53,866 (technical myclobutanil, 84.5% pure) was tested in a 2-generation reproduction study with male and female CRL:CD(SD)BR rats. The rats were obtained from Charles River Breeding Laboratories, Kingston Facility, Stone Ridge, NY. Twenty-five animals/sex/dose group received 0, 50, 200 or 1000 ppm in the diet throughout the study (0, 2.5, 10 or 50 mg/kg/day by standard conversion factor). The animals were mated on a one to one ratio with the F_0 parental animals and were given test diets for 8 weeks before they were mated. Selection of the parents for the F_1 generation was made when the pups were 25 days of age, and the mated animals in the study were approximately 81 days of age at mating.

At 200 ppm, centrilobular hepatocellular hypertrophy was observed in the P_2 males. This was supported by slight but statistically significant increases in liver weights in males: (114% absolute, 107% relative for P_1 and 107% absolute and 104% relative for P_2). At 1000 ppm, centrilobular hepatocellular hypertrophy was observed in both sexes in the P_1 and P_2 generations. These were again supported by slight but statistically significant increases in liver weights: males: (113.6% absolute, 114% relative for P_1; 107% absolute, 113% relative for P_2); females: (109% absolute,

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109% relative for P_1; 106% absolute, 108% relative for P_2). Therefore, **the parental (systemic) toxicity LOEL is 200 ppm and the parental (systemic) toxicity NOEL is 50 ppm** based on hepatocellular hypertrophy and increases in liver weights.

At 1000 ppm, an increase in the number of stillborn or % born dead was observed in both generations (4.9 - 5.3% versus 0 - 1.9% in controls). In addition, multifocal or diffuse testicular atrophy was observed in males in the P_2 generation. Increased necrotic spermatocytes/spermatids or decreased spermatozoa and atrophy of the prostate were also observed in these animals. Therefore, **the reproductive toxicity LOEL is 1000 ppm and the reproductive toxicity NOEL is 200 ppm** based on an increased incidence in the number of stillborns and atrophy of the testes and prostate.

At 1000 ppm, it appears that there was a decrease in pup weight gain during lactation (83.3% to 89.7% of the controls). Therefore, the **developmental toxicity LOEL is 1000 ppm and the developmental toxicity NOEL is 200 ppm** based on a decrease in pup body weight gain during lactation.

This study is classified as Core Guideline and satisfies the regulatory requirement for a multigeneration reproduction study in the rat (83-4).

This is a supplemental DER to add sufficient details to the previously written DER for an adequate assessment of reproductive toxicity.

I. PROTOCOL

A. Materials

1. Test Material

Chemical Name: α-butyl-α-4-chlorophenyl-1-H-1,2,4-triazole-1-propanenitrile

Description: brown solid
Batch #(s), Other #(s): TD. # 83-155, Lot # LSPL 83/0017E
Purity: 84.5%
Source: Rohm and Haas
Vehicle: Acetone

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2. Test Animals:

Species and Strain (sexes): Male and female CRL:CD(SD)BR rats

Age: 25 days old upon receipt
Source(s): Charles River Breeding Laboratories, Kingston Facility, Stone Ridge, NY

The rats were acclimated for a period of 14 days before they were placed into the study.

3. Diet preparation: RH-53,866 was heated in a water bath to 50 - 60°C until melted; stirred until homogeneous and separated into aliquots. These were all stored in vials until use. Each week, one vial was heated until melted and stirred. For each dose level, the appropriate amount was weighed out and mixed with acetone. The acetone-pesticide solution for each dietary level was mixed with 1.0 kg of untreated feed in order to evaporate the acetone. The premix was then added to appropriate amounts of feed and mixed again.

Frequency of preparation: Weekly.

Storage conditions: Not stated, assumed room temperature.

Stability Analyses: Each week one extra food cup was filled with each dietary level and left on top of a cage bank in the study room during the treatment week. At the end of each week, each sample was placed into a plastic bag and submitted for analysis of active ingredient for a stability analysis.

Homogeneity Analyses: Samples from the top, middle and bottom of each diet mix from the first time the diets were prepared were collected and analyzed for uniformity of mixing.

Concentration Analyses: Not stated.

B. Procedures and Study Design

1. Mating: One male was caged with 1 female from the same test group until sperm cells were observed in vaginal smears taken daily during the mating period. If sperm were not found after 10 days' observation, the first male was removed and days later was replaced by another male from a group of males with proven fertility in the same test group. If two attempts at mating were unsuccessful the report stated that no further matings were tried.

After successful mating, each pregnant female was individually placed into a cage with a solid bottom and Alona-Dri® bedding where they were kept through gestation and lactation.

2. Mating schedule: The F_0 parental animals were given test diets for a minimum of 8 weeks before they were mated, and the F_1 parental animals were not mated until 8 weeks after they were selected from the F_{1A} litters. Selection of parents for the F_1 generation was made when the pups were 25 days of age, and the mated animals in the study were approximately at least 81 days (11.6 weeks) of age at mating.

3. Animal assignment: F_0 animals were randomly assigned to test groups as follows:

Test groups		Dose	Animals per group **	
No.	Designation	(ppm) *	Males	Females
1	Control	0	25	25
2	Low (LDT)	50	25	25
3	Mid	200	25	25
4	High (HDT)	1000	25	25

* Diets were administered from the beginning of the study until the animals were sacrificed.

** The same number of animals were picked from the F_1 litters as parents for the F_2 generation.

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II. REPORTED RESULTS

A. Parental animals

Body weight and food consumption:

Observation and study day	Dose group: Control	Low	Mid	High
F_0 Generation Males - Pre-mating				
Mean body weight (g)				
0	255.2	258.9	260.3	261.0
49	430.8	439.0	443.2	431.3
Mean weight gain (g) Days 0 - 49	175.6	180.1	182.9	170.3
Mean food consumption (g/rat/day)				
0	25.97	26.35	25.52	26.12
7	26.98	26.72	25.99	25.68*
49	26.67	27.63	27.67	26.63
F_0 Generation Females - Pre-mating				
Mean body weight (g)				
0	171.6	168.9	174.7	170.5
49	260.3	250.1	256.6	249.5
Mean weight gain (g)[a] Days 0 - 49	88.7	81.2	81.9	79.0 (89.1%)
Mean food consumption (g/rat/day)				
0	18.23	18.20	18.71	18.21
7	19.35	9.18	19.14	18.41*
49	19.53	20.34	20.35	19.87

* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.
[a]Body weight gains calculated by EPA reviewer: no statistics conducted.

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Observation and study day	Dose group			
	Control	Low	Mid	High
F_1 Generation Males - Pre-mating				
Mean body weight (g)				
0	199.5	207.4	191.8	183.9
56	453.5	463.9	449.9	415.6*
Mean weight gain (g)[a]				
0 - 56	254.0	256.5	258.1	231.7
Mean food consumption (g/rat/day)				
7	24.77	25.11	24.67	23.72
14	26.36	26.03	25.98	26.09
56	27.48	27.31	27.55	26.37
F_1 Generation Females - Pre-mating				
Mean body weight (g)				
0	154.5	158.5	153.6	149.0
56	266.6	272.5	270.8	259.8
Mean weight gain (g)[a]				
0 - 56	112.1	114.0	257.2	110.8
Mean food consumption (g/rat/day)				
7	18.01	17.81	18.54	17.44
14	18.58	18.35	19.02	18.24
56	19.70	19.93	20.08	20.04

* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.
[a]Body weight gains calculated by EPA reviewer: no statistics conducted.

Selected group mean body weights and food consumption values for pregnant or nursing dams were summarized in the report as follows:

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Observation and study time	Dose group Control	Low	Mid	High
F_0 Generation - Litter A				
Mean body weight (g)				
Day 1 of gestation	256	258	258	255
Day 20 of gestation	378	386	388	381
Day 1 of lactation	296	304	298	293
Day 21 of lactation	315	319	316	314
Mean body weight gain (g)				
Days 1-20 of gestation	122	128	130	125
Day 1-21 of lactation	19	15	18	21
F_0 Generation - Litter B				
Mean body weight (g)				
Day 1 of gestation	291	297	300	298
Day 20 of gestation	413	429	432	423
Day 1 of lactation	334	341	335	327
Day 21 of lactation	340	355	341	350*
Mean body weight gain (g)				
Days 1-20 of gestation	122	135	133	134
Day 1-21 of lactation	6	14	6	23
F_1 Generation - Litter A				
Mean body weight (g)				
Day 1 of gestation	264	270	264	254
Day 20 of gestation	395	398	391	376
Day 1 of lactation	315	311	309	291
Day 21 of lactation	336	338	329	324
Mean body weight gain (g)				
Days 1-20 of gestation	130	129	127	122
Day 1-21 of lactation	21	27	20	33

Observation and study time	Dose group Control	Low	Mid	High
F_1 Generation - Litter B				
Mean body weight (g)				
Day 1 of gestation	305	308	311	294
Day 20 of gestation	434	436	442	423
Day 1 of lactation	345	339	348	327
Day 21 of lactation	372	370	374	356
Mean body weight gain (g)				
Days 1-20 of gestation	129	128	131	128
Day 1-21 of lactation	27	31	26	29

* Statistically significantly different from control, $p<0.05$.
* Statistically significantly different from control, $p<0.01$.

Reproductive performance:

Observation	Dose group Control	Low	Mid	High
F_0 Generation - Litter A				
Median precoital interval (days)	3.1	2.8	2.6	3.3
Females				
Number mated	25	25	25	25
Number sperm positive	25	25	25	25
Median gestation interval (days)	21.8	21.8	21.8	22.1
Number of litters	23	24	22	20
Mean litter size (Day 0)[e]	13.6	12.6	13.3	11.7
Mean litter size (Day 4) (pre-cull)	13.5	12.4	13.2	11.9[b]
Mean litter size (Day 7)	9.8	9.3	9.5	9.3
Mean litter size (Day 14)	9.8	9.3	9.5	9.3
Mean litter size (Day 21)	9.8	9.3	9.5	9.3
Number of live pups (Day 0)	313	302	293	233
Number of live pups after cull	226	224	209	177
Number of live pups (Day 21)	225	224	209	177
Number of pups born dead	3	4	9	12*
(%)	(0.9)	(1.3)	(3.0)	(4.9)
Viability index[c]	98.4	97.1	96.4	92.7*
Lactation index[d]	99.6	100.0	100.0	100.0
Mean pup weight (g) (Day 0)[a]	6.0	6.1	6.2	6.3
Mean pup weight (g) (Day 21)	45.7	45.9	44.4	41.9
Mean pup weight gain (Days 0 -21)	39.7	39.8	38.2	35.6 (89.7%)

* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.
[a]Including pups that were born dead.
[b]One less dam at day 4.
[c]Viability index = # alive at 4 days/total born
[d]Lactation index = # alive at 21 days/# alive after culling on day 4.
[e]Litter sizes and pup weight gain calculated by EPA reviewer; statistics not conducted.

Observation	Dose group			
	Control	Low	Mid	High
F_0 Generation - Litter B				
Median precoital interval (days)	2.4	2.6	2.7	2.1
Females				
Number mated	25	25	25	25
Number sperm positive	25	23	25	22
Median gestation interval (days)	21.9	21.8	22.0	21.9
Number of litters (Day 0)	22	22	23	23
Mean litter size (Day 0)[d]	13.0	13.0	13.3	13.5
Mean litter size (Day 4) (pre-cull)	11.7	11.3	11.0	12.3
Mean litter size (Day 7)	9.4	9.0	9.1	9.2
Mean litter size (Day 14)	9.3	9.0	9.0	9.1
Mean litter size (Day 21)	9.3	9.0	8.9	9.1
Number of live pups (Day 0)	287	286	306	311
Number of live pups after cull	208	200	202	211
Number of live pups (Day 21)	204	198	196	210
Number of pups born dead	0	6	9*	16*
(%)	0.0	2.1	2.9	4.9
Viability index[b]	89.9	85.3	77.1	86.2
Lactation index[c]	98.1	99.0	97.0	99.5
Mean pup weight (g) (Day 0)[a]	5.9	6.0	6.1	5.9
Mean pup weight (g) (Day 21)	46.6	45.6	46.2	42.2
Mean pup weight gain (Days 0-21)	40.7	39.6	40.1	36.3 (89.2%)

* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.
[a]Including pups that were born dead.
[b]Viability index = # alive at 4 days/total born
[c]Lactation index = # alive at 21 days/# alive after culling on day 4.
[d]Litter sizes and pup weight gain calculated by EPA reviewer; statistics not conducted.

Observation	Dose group			
	Control	Low	Mid	High
F_1 Generation - Litter A				
Median precoital interval (days)	2.2	3.1	2.8	3.0
Females				
Number mated	25	24	25	25
Number sperm positive	25	23	23	22
Median gestation interval (days)	21.7	21.7	21.9	22.2
Number of litters (Day 0)	23	23	24	20
Mean litter size (Day 0)[e]	13.7	13.7	13.7	9.8
Mean litter size (Day 4) (pre-cull)	12.0	11.7	11.9	10.7[d]
Mean litter size (Day 7)	9.1	9.3	9.4	9.4
Mean litter size (Day 14)	9.5[d]	9.3	9.4	9.4
Mean litter size (Day 21)	9.5	9.3	9.4	9.4
Number of live pups (Day 0)	314	314	314	216
Number of live pups after cull	209	215	216	169
Number of live pups (Day 21)	208	213	216	169
Number of pups born dead	6	3	1	13*
(%)	1.9	0.9	0.3	5.7
Viability index[b]	86.8	84.9	86.7	84.6
Lactation index[c]	99.5	99.1	100.0	100.0
Mean pup weight (g) (Day 0)[a]	5.8	6.1	6.2	6.2
Mean pup weight (g) (Day 21)	46.3	46.6	44.6	40.2
Mean pup weight gain (Days 0 - 21)	40.8	40.5	38.4	34.0 (83.3%)

* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.
[a]Including pups that were born dead.
[b]Viability index = # alive at 4 days/total born
[c]Lactation index = # alive at 21 days/# alive after culling on day 4.
[d]Fewer number of females with litters than previous observation.
[e]Litter sizes and pup weight gain calculated by EPA reviewer; statistics not conducted.

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Observation	Dose group			
	Control	Low	Mid	High
F_1 Generation - Litter B				
Median precoital interval (days)	3.0	3.4	2.9	4.4
Females				
Number mated	25	24	25	25
Number sperm positive	24	21	25	21
Median gestation interval (days)	21.7	22.0	21.8	21.7
Number of litters (Day 0)	23	22	25	17
Mean litter size (Day 0)[d]	15.2	14.5	13.6	12.8
Mean litter size (Day 4) (pre-cull)	14.9	13.9	14.1[e]	12.9[e]
Mean litter size (Day 7)	10.0	9.6	10.0	9.6
Mean litter size (Day 14)	10.0	9.6	10.0	9.6
Mean litter size (Day 21)	10.0	9.6	10.0	9.6
Number of live pups (Day 0)	349	319	341	218
Number of live pups after cull	230	207	240	155
Number of live pups (Day 21)	220	202	239	144
Number of pups born dead	5	6	3	12
(%)	1.4	1.8	0.9	5.3
Viability index[b]	96.9	94.2	98.5	90.8
Lactation index[c]	95.7	97.6	99.6	92.9
Mean pup weight (g) (Day 0)	6.0	5.9	6.1	5.8
Mean pup weight (g) (Day 21)	46.5	48.1	46.0	41.8
Mean pup weight gain (Days 0 - 21)	40.5	42.2	39.9	36.0 (88.9%)

* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.
[a]Including pups that were born dead.
[b]Viability index = # alive at 4 days/total born
[c]Lactation index = # alive at 21 days/# alive after culling on day 4.
[d]Litter sizes and pup weight gain calculated by EPA reviewer; statistics not conducted.
[e]Fewer dams with litters than in previous observation.

CASWELL FILE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

004936

FEB 11 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Systhane or Myclobutanil (RH-53,866 or RH-3866)
EPA Identification Nos. 4G-3149/707-ROG/707-EUP-RNL
CASWELL No. 723K

FROM: Jane E. Harris, Ph.D. JEH 2/4/86
Section Head, Section VI
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Henry M. Jacoby, PM 21
Fungicide-Herbicide Branch
Registration Division (TS-767C)

THRU: Theodore M. Farber, Ph.D., Chief
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Applicant: Rohm & Haas Company
Philadelphia, PA

Submission Purpose

Review the following: Two-generation reproduction study in rats; interim report on testicular pathology in two-year chronic rat and mouse oncogenicity studies, and preliminary findings in one-year dog study; Accession Nos. 073522, 073805, 073806, and 073807; also, review adequacy of labeling changes.

Recommendation

The highest dose (1000 ppm or about 80 mg/kg bwt) of the 2-generation reproduction study was associated with testicular atrophy in the second (P_2) generation, supporting a NOEL of 200 ppm (16 mg/kg bwt). Seminiferous tubular atrophy of the testes was also observed at an increased incidence in the

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800 ppm dose level at 12 and 17 months in the interim sacrifices of the 2-year chronic rat feeding study, supporting a similar NOEL to the reproduction study of 200 ppm. No treatment-related increases in testicular atrophy were identified in the 1-year dog study at dietary levels up to 1600 ppm (40 mg/kg bwt) or at 12 months in the mouse oncogenicity study at dietary levels up to 500 ppm (75 mg/kg bwt).

The overall NOEL for the 2-generation reproduction study is 50 ppm (or 4 mg/kg bwt) based on increased absolute and relative liver weights and increased centrilobular hepatic hypertrophy in male rats at 200 ppm (or 16 mg/kg bwt). The 2-generation reproduction study in rats is classified as Core Guideline.

In view of these testicular lesions observed in rats in the reproduction and chronic studies, the registrant has submitted an amendment of the label requiring new protective clothing (long trousers, long-sleeved shirts), impervious gloves, and splash goggles during all methods of mixing, loading, applying, or handling. This new labeling at the present time appears appropriate for purposes of the Experimental Use Permit (EUP).

I. Two-Generation Reproduction Study (Report No. 84R-117; August 21, 1985)

Chemical: RH-3866 or RH-53,866 Technical 84.5% ai: α-butyl-α-4-chlorophenyl-1-H-1,2,4-triazole-1-propanenitrile.

Materials and Methods (See Appendix I for details).

Two matings per generation were performed with 25 rats, Sprague-Dawley (Crl:CD(SD)BR) per sex per group exposed to RH-53,866 at 0, 50, 200, and 1000 ppm. P_1 rats were exposed to compound for 8 weeks prior to mating and through the reproductive phase. The P_2 rats were exposed continuously to compound from conception to at least 8 weeks after weaning and through the reproductive phase.

Clinical signs in the P_1 and P_2 rats were monitored daily. Food consumption and body weights were monitored weekly until cohabitation. Presumed pregnant females were weighed on day 0, 6, 15, and 20 of gestation and lactating dams were weighed on day 0, 4, 7, 14, and 21 of lactation. Necropsies were performed on all P_1 and P_2 animals found dead or killed *in extremis*. Following weaning of the F_{1b} and F_{2b} litters, all surviving P_1 and P_2 rats were subjected to full necropsies and liver to body weight determinations were recorded.

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Histopathologic examinations of P_1 and P_2 animals were performed on all gross lesions, on livers, and male reproductive organs in all dose groups. Female reproductive organs were histopathologically examined in the controls and high dose group.

Clinical signs in pups were monitored daily. Body weights, progeny counts, and sex determinations were recorded on lactation day 0, 4, 7, 14, and 21. Pups were randomly culled to 10 pups (5 of each sex) on day 4 and weaned on day 22 of lactation. Necropsies were performed on all F_1 and F_2 pups found dead after 14 days of age.

Results

Diet Analysis and Intake - The average concentration of active ingredient in the samples ranged from 102 percent to 112 percent of the theoretical concentration with an overall mean of 106 percent. The average overall exposure to RH-53,866 in treated groups prior to mating for each generation was calculated on a daily basis to be 4 mg/kg bwt, 16 mg/kg bwt, and 80 mg/kg bwt, for dietary levels 50 ppm, 200 ppm, and 1000 ppm, respectively.

Parental Toxicity

A few deaths occurred at random during the study across all dose groups and were considered unrelated to treatment. Body weight gain for dams was not significantly altered with treatment during the premating, gestation, and lactation periods. Males of the P_2 generation showed 9 percent lower weight gain throughout the 8 weeks prior to mating. A slight reduction in food consumption occurred at the highest dose prior to mating and may reflect a modest degree of poor palatability for the treated chow in both the P_1 and P_2 animals.

Gross Pathology and Liver Weights

P_1 and P_2 animals showed increased absolute liver weights and liver to body weight ratios at the highest dose level in both sexes and at 200 ppm in male rats (see table below).

Liver Weights

	P_1 Abs. Liver Wt (g)	Rel($x10^4$) Liver/Body Wt	P_2 Abs. Liver Wt (g)	Rel($x10^4$) Liver/Body Wt
Male				
Control	12.77	247	18.2	314
200 ppm	14.56*	264*	19.5*	328*
1000 ppm	14.51*	281*	19.5*	356*
Female				
Control	8.59	289	12.5	367
200 ppm	9.04	295	12.9	373
1000 ppm	9.35*	314*	13.2*	397*

*$p < 0.05$

Gross necropsy also showed an increase in small or flaccid testes at the HDT (8/25) in comparison with controls (0/25) in the P_2 but not P_1 rats.

Histopathology

P_1 and P_2 rats of both sexes at the HDT and males at the 200 ppm level showed evidence of centrilobular hepatic hypertrophy.

Incidence of Centrilobular Hepatic Hypertrophy

	P_1	P_2	
	1000 ppm	1000 ppm	200 ppm
Females	8/25	4/25	
Males	10/25	18/25	2/25

No evidence of hepatic hypertrophy was demonstrated in other treatment groups or controls of the P_1 and P_2 rats (0/25 per group).

Consistent with the gross pathology of small and flaccid testes in the HDT of the P_2 rats, histopathologic examination demonstrated multifocal or diffuse atrophy of the testes in this group at an increased incidence as compared with controls (see below). The high dose males of the P_2 rats also showed increased incidences of epididymal lesions and atrophy of the prostate.

Incidences of Reproductive Lesions in Males

	Dietary Levels				
	Con	Low	Mid	High	
Multifocal or Diffuse Testicular Atrophy	3/25	5/25	5/25	11/25	P_2
	3/25	3/25	3/25	3/25	P_1
Epididymal Lesions					
Necrotic spermatocytes/ spermatids or	2/25	3/25	3/25	13/25	P_2
Decreased spermatozoa	1/25	2/25	0/25	3/25	P_1
Prostate					
Atrophy	2/25	1/25	0/25	11/25	P_2
	2/25	0/25	1/25	2/25	P_1

Reproductive Toxicity

With respect to reproductive parameters, the P_2 generation showed some equivocal increases in reproductive toxicity at the highest dose level evidenced by a slight decrease in the percentage of dams littering (86% vs. 96% in controls) in the P_{2b} mating and a decreased number of pups born per litter (11.4 vs. 13.8 in controls) in the P_{2a}. Reproductive toxicity was more clearly evidenced in the four matings at the high dose (1000 ppm) by an increased number of stillborn or percent born dead.

Number Born Dead (Percent Born Dead)

	Control	1000 ppm
P_{1a}	3 (0.9%)	12* (4.9%)
P_{1b}	0 (0.0%)	16* (4.9%)
P_{2a}	6 (1.9%)	13* (5.7%)
P_{2b}	5 (1.4%)	12 (5.3%)

*$p < 0.05$

Pup Toxicity

Although pup weight was not significantly different between groups at birth, by day 4 in the F_1 pups and by day 7 in the F_2 pups, significant decreases in pup weights were observed in the 1000 ppm group in comparison with controls. The depression of weight gain in the 1000 ppm group in comparison with controls increased as pups matured through day 21 of lactation (see tables 6A-D, in Appendix II).

Discussion and Conclusions

At the highest dose tested, 1000 ppm, reproductive toxicity was most evidenced by an increase in the number of stillborns in each of the two matings per generation. More general toxicity to the parental generations was an increase in absolute and relative liver weights at 200 and 1000 ppm in males and 1000 ppm in females in both P_1 and P_2 rats. These increases in liver weights correlate with the finding of centrilobular hepatic hypertrophy at 1000 ppm in both sexes of P_1 and P_2 rats and in males at 200 ppm in the P_2 rats, suggestive of possible microsomal enzyme induction. P_2 males in the high dose group, which were also exposed to RH-53,866 in utero and during lactation, also showed testicular atrophy, which may as a secondary response to depressed testosterone levels, result in increased atrophy of the prostate and epididymal lesions. P_2 males also demonstrated a decreased body weight gain (9%) prior to mating, suggestive of a greater toxic response to RH-53,866 than the P_1 rats. Finally, weight gain of pups progressively decreased from day 4 through day 21 of lactation in the high dose group in comparison with controls.

The study supports a reproductive NOEL of 200 ppm (16 mg/kg bwt/day) and a LEL of 1000 ppm (80 mg/kg bwt/day), based on: testicular, epididymal, and prostatic atrophy in P_2 males, and in both generations, increased numbers of stillborns, and decreased weight gain in pups during lactation. General systemic toxicity evidenced by increased liver weights and centrilobular hepatic hypertrophy in males at 200 ppm (16 mg/kg bwt/day) support a systemic NOEL of 50 ppm (4 mg/kg bwt/day).

Core Classification: Guideline